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The Effect of Ten Days of Heat Acclimation on Exercise Capacity during Acute Altitude Exposure (4350 m)

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THE EFFECT OF TEN DAYS OF HEAT ACCLIMATION ON EXERCISE CAPACITY DURING ACUTE ALTITUDE EXPOSURE (4350 M)

BY

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The Effect of Ten Days of Heat Acclimation on Exercise Capacity during Acute Altitude Exposure (4350 m)

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ABSTRACT

Acclimation to one environmental stressor may enhance adaptations to various other stressors in humans; this phenomenon has been described as cross-tolerance. Approaches to induce altitude acclimation in a relatively short time frame are needed, thus the purpose of our study was to examine the effect of heat acclimation on exercise capacity during acute altitude exposure (4350 m). Eight trained men residing at approximately 1600 m performed tests of maximal aerobic capacity (VO_{2max}) at 1600 m and 4350 m, a 16 km time-trial (TT) (4350 m), and a heat tolerance test (1600 m) before and after a 10 d heat acclimation (HA) protocol (40°C, 20% RH). Blood samples were taken at rest pre- and post-HA to estimate changes in plasma volume with HA. Heat acclimation was achieved, as indicated by a significantly lower post-exercise heart rate ($p < 0.01$) and rectal temperatures ($p = 0.42$) on the last versus the first day of HA. Heat acclimation did not alter plasma volume ($p = 0.61$, 1.8 ± 9.9%) or VO_{2max} at 1600 m (4.02 ± 0.58 L/min vs. 4.03 ± 0.43 L/min, $p = 0.88$) or at 4350 m (3.40 ± 0.33 L/min vs. 3.46 ± 0.38 L/min, p

= 0.48). Time-trial performance was improved by 27.6 sec after HA ($p = 0.06$), which was revealed to be a 95% worthwhile effect according to magnitude based statistics. Our findings do not support the use of HA to significantly improve VO_{2max} during acute altitude exposure. However the improved TT performance suggests that HA was beneficial. Further research with more subjects needs to be conducted to elucidate the use of HA to enhance adaptation to acute altitude exposure.

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I would like to thank Roy Salgado for his assistance with subject recruitment and data collection.

Chapter 1

Introduction

There is growing interest in the participation in mountain recreation and endurance events at high altitude, as well as a greater number of US troops being deployed to high altitude terrain. Individuals not properly acclimatized/acclimated to high (2300 m) and extreme (8848 m) altitude will experience a reduction in both submaximal and maximal exercise performance (6, 17, 34, 48). The observed reduction in performance is due to the decline in arterial oxygen pressure, oxygen saturation of hemoglobin, and arterial oxygen content (17, 40). During altitude exposure, a given absolute workload requires a greater relative exercise intensity when compared to sea level (35, 49). Cycling time trial performance during intermittent altitude exposure at 4300 m over several weeks can take 60-70% longer compared to sea level (3, 16), and can be reduced by 40% during acute altitude exposure (4300 m) (4). However, others have shown a smaller decrease in submaximal endurance performance following 2-3 weeks of high altitude exposure (27, 33). Young et al. (50) reported that chronic (15 days) altitude exposure while residing at 4300 m did not significantly change maximal aerobic capacity (VO_{2max}), similar to a study by Faulkner et al. (12) examining 6 weeks of training at 2300 m.

Athletes and military personnel ascending to high altitude may not be given ample time or means to properly acclimatize/acclimate to a hypoxic environment. Therefore, alternate training and/or acclimation methods have been examined as a means to improve

or maintain exercise performance prior to ascending to high altitude environments. Previously used methods include: “live high: train low” (30), altitude tents, and more recently, intermittent altitude exposure (IAE) (3). However, these methods can be time consuming and require equipment that is expensive or of limited availability.

It has been suggested that acclimation to one environmental stressor could lead to enhanced adaptations to various other stressors (15). This phenomenon has been described as cross-tolerance and involves the activation of common protective pathways (29). An early study (23) examined heat acclimated (HA) mice at 36-37°C for either 10 or 14 days, and results showed that HA mice survived longer before drowning. Cross-tolerance between cold and hypoxia exposure in rats was examined by exposing rats to either a cold or hypoxic environment. Those that were chronically exposed to the cold environment had less tolerance to acute hypoxia than those that were exposed to hypoxia alone (15). Consequently, cross-tolerance from one environment to another may not always be beneficial for animals and humans. More recently, researchers have continued to support the thought that cross-tolerance may exist between HA and a low oxygen environment in the heart (24), as HA may elicit a protective response to a low oxygen environment. Following HA in rats, the hearts diastolic pressure-volume curve is shifted to the right, allowing the ventricle to have a larger filling volume without further increases in filling pressure (24). Improved cardiac function in HA rat heart is due to increased compliance, contractility, and reduced stiffness of the heart (26). If a cross-tolerance model could be shown to be beneficial for humans, it could lead to the development of protocols to be used as an alternative means to prepare for exercise at altitude and perhaps maintenance of exercise performance at altitude.

Intermittent Altitude Exposure

Simulated altitude training has been used to induce performance enhancements for athletes and military personnel. Prolonged (~ 4 weeks) altitude exposure (2500 m) in conjunction with training at a lower altitude (1250 m) enhanced exercise performance in competitive athletes (9, 30). Recently, intermittent altitude exposure (IAE) has been implemented as an alternative to chronic altitude acclimatization as a potential strategy to improve exercise performance at altitude (3, 4, 8). Mountaineers improved exercise performance following 17 days of low intensity exercise combined with exposure to intermittent hypobaric hypoxia exposure (8). Three weeks of IAE at 4300 m in combination with a training protocol improved cycling time-trial performance at altitude (+21%) with an increase in resting oxygen saturation of hemoglobin (SaO_2) (3). Beidleman et al. (4) concluded that 7 days of IAE (4 hr/day) was adequate to improve time-trial cycling performance at altitude (4300 m) by 16% when compared to baseline (pre-IAE) at 4300 m. Furthermore, reductions in heart rate (9-20 b/min), VO_2 (1.5-1.6 L/min), RPE, and a higher SaO_2 (3-4%) were also observed, suggesting that the subjects worked at a lower percentage of VO_{2max} at 4300 m. Interestingly the 16% improvement in time-trial performance was accomplished after only spending a total of 28 hrs at 4300 m. These data contradict earlier work showing a similar increase in time-trial performance after spending 128 hrs at 4300 m (16). Therefore, the above findings support the use of IAE to elicit physiological adaptations that are similar to those observed in previous studies following chronic altitude acclimatization. IAE can be used as an efficient method to improve time-trial performance at altitude (4). Other methods to induce acclimation to high altitude in a relatively short timeframe are still needed, as it is not practical for many

athletes and soldiers to get access to facilities providing IAE. Therefore, there is a need to develop other strategies that may preserve or improve performance during acute altitude exposure and are practical to employ in situations that require short notifications or rapid deployment.

Decreased Plasma Volume during High Altitude Exposure

Chronic altitude exposure has been associated with a reduction in plasma volume (20). Convective heat transfer from the skin to the atmosphere during high altitude exposure is reduced, however evaporative heat loss is enhanced due to the reduced barometric pressure (18, 19). For example, Hannon et al. (20) found a 20% reduction in plasma volume while living at Pikes Peak for 14 days. Exercise at altitude distributes a larger portion of cardiac output to the working muscles (45), thus an increased amount of plasma water may be shifted to the working muscles resulting in a reduction in PV (14). Alexander and Grover (1) concluded that 10 days of altitude exposure (3100 m) reduced PV by 20% and may contribute to reduced left ventricular filling and venous return at rest and during exercise.

Heat Acclimation and Plasma Volume Expansion

Individuals become HA following repeated exposures to high ambient temperatures that elevate skin and core temperatures sufficiently in order to elicit perfuse sweating. Improvements in exercise performance following HA in humans result from several physiological adaptations that include reduced oxygen uptake and blood lactate at a given submaximal power output (42, 51), muscle glycogen sparing (13, 51), PV expansion (2, 44), and improved myocardial efficiency (25). It has been proposed that PV

expansion following HA results from: 1) increased sodium and water retention, which increase the extracellular fluid and plasma volume; 2) increases in circulating protein increase oncotic pressure and move water from the interstitium to plasma; and 3) enhanced dilation of the veins in response to the heat, decreasing post-capillary resistance and increasing the net fluid movement into the plasma (2, 21, 41, 44). PV expansion following HA can improve aerobic and thermoregulatory capacities (36, 37, 44). However, it is important to note that PV expansion is not always observed following HA (2, 11). The magnitude of PV expansion depends on whether the individual is participating in passive or active HA, the individual's hydration and fitness level, as well as current HA status (41). In one study, 10 days of HA increased VO_{2max} and time-trial performance, leading authors to believe that this could be due to the observed expansion in PV (+6.5%) (31). An increase in PV could also elicit improved myocardial efficiency and ventricular compliance (26) resulting in an increase in end-diastolic volume and thus VO_{2max}. Conversely, Heled et al. (22) found no change in VO_{2max} following 12 d of HA, however the authors did not measure PV. Nielsen et al. (36) found no significant differences in VO_{2max} following HA despite increases in PV (+13.1 %) as well as cardiac output and stroke volume. Yet, the authors did not explain why there was no change in VO_{2max} in their subjects even in the presence of an increased O_2 delivery and no difference in leg blood flow at 10 min of exercise and at exhaustion.

Plasma Volume and Exercise Performance

Coyle et al. (10) examined the effects of PV expansion on exercise performance and VO_{2max}. A larger PV expansion (500-700 mL) did not improve performance or VO_{2max}, but a smaller expansion of PV (200-300 mL) increased VO_{2max}

and time to exhaustion by 3.7% and 6.8%, respectively, and increased stroke volume by 15% during submaximal cycling. The authors concluded that a larger PV expansion (500-700 mL) might not be optimal for improving exercise performance due to increased hemodilution. Acute plasma volume expansion can lead to a significant reduction in hemoglobin concentration and hematocrit causing a reduction in arterial oxygen content. However, a smaller expansion in PV may contribute to increases in stroke volume, cardiac output, and a higher peak ventricular end-diastolic volume during exercise (10). Others have examined the effect of a 400 mL PV expansion on time-trial performance in trained cyclists. Results showed that mean power output increased (+10%), as well as performance (+11%) compared to the control trial (32). However, the observed hemodilution did not negatively influence $VO_{2\ peak}$ or cycling time to exhaustion (5), thus a 14% increase in PV resulted in a 6% increase in VO_{2peak} despite an 8% reduction in hemoglobin concentration (5). However, others have found no increase in maximal cardiac output following PV expansion at sea level (28, 46). After a +13% expansion of PV, TT performance was not improved during exercise in the heat (47).

Others have examined the effect of PV expansion on exercise capacity and maximal cardiac output in acclimatized lowlanders after spending 9 weeks at 5260 m (7). A one-liter expansion of PV had no effect on VO_{2max} or maximal cardiac output in this population. However, Robach et al. (38) examined 31 days of decompression in a hypobaric chamber at an altitude equivalent to greater than 4500 m and ascending to an altitude of 8848 m during the last two days of the exposure. Incremental VO_{2max} tests were performed with and without PV expansion at sea level, 6000 m, and upon return to sea level. The authors concluded that VO_{2max} improved by 9% at altitude (6000 m) in

acclimated subjects following acute PV expansion, however PV expansion had no effect on VO_{2max} at sea level or upon return to sea level.

Heat Acclimation and Aerobic Capacity

Thermal stress results in increased blood flow to the periphery, which may reduce blood flow perfusing the working muscles and/or reduce venous return and cardiac output. Sawka et al. (43) examined effects of HA on VO_{2max} obtained in a moderate (21°C) versus a hot (49°C) environment. Data showed that HA increased VO_{2max} by 4% in both environments, which was explained by a greater fraction of cardiac output available to perfuse working muscles (39). However, others have found no difference in VO_{2max} following HA (13).

It is unknown whether prior HA in humans could alter exercise performance during exposure to high altitude. The novel use of a HA model prior to deployment or an athletic competition could serve as a possible alternative for individuals preparing to ascend to high altitudes. This approach may help maintain or improve performance during high altitude exposure. The proposed mechanism/s for this model include an expansion of PV, as well as an increase in VO_{2max}, thus leading to greater venous return and resultant maintenance of cardiac output during exercise at altitude.

Study Purpose and Hypotheses

The purpose of this study is to determine whether 10 days of heat acclimation will lead to an expansion in plasma volume and an increase in VO_{2max} in trained men. We also propose that 10 d of HA will enhance exercise performance during a16 km time-trial at altitude (4350 m).

Purpose of this Study

1. To demonstrate if 10 days of heat acclimation will lead to an increase in VO_{2max} and time-trial performance during acute altitude exposure (4350 m). We propose that an expansion of plasma volume will ultimately lead to an improved exercise capacity during acute altitude exposure. This specific model has never been tested on humans, thus, if successful, it could be used as an alternative model to prepare for exercise or occupational work at high altitude.

Hypotheses

1. Ten days of heat acclimation will cause an increase in plasma volume in trained men.

   Heat acclimation can induce an expansion in plasma volume in humans (36, 44).

2. Ten days of heat acclimation will lead to an increase in VO_{2max} at 4350 m, which may be mediated by the expansion in plasma volume.

   A moderate expansion in plasma volume (200-300 mL) increased VO_{2max} in untrained men (10). Ten days of heat acclimation increased VO_{2max} and plasma volume (31).

3. Ten days of heat acclimation will lead to an improvement in 16 km time-trial performance during acute altitude exposure (4100 m).

   Heat acclimation has been shown to improve submaximal oxygen requirements (42), thus during exercise, a reduction in the relative $\%VO_{2max}$ can lead to an increase in submaximal performance (31). Lorenzo et al. (31) examined trained cyclists following 10 consecutive days of exercise at 50% VO_{2max} in a

thermoneutral environment and the same protocol was also performed in a hot environment. The researchers observed no significant difference between pre-to-post VO_{2max} and TT performance in the control group. However, there was a significant increase in PV, VO_{2max} and TT performance in the treatment group following 10 days of HA.

Limitations

1. Acute exposure to altitude (4350 m) may not be applicable to athletes and military personnel, as these individuals typically travel to altitude for prolonged and/or chronic periods of time.
2. If plasma volume is elevated, hemodilution can occur, ultimately reducing the concentration of red blood cells, thus without measurement of cardiac output and stroke volume we can only speculate that any increase in VO_{2max} resulted from an increase in plasma volume.
3. In this study we will not be using a control group, and therefore if we do find significant differences we can only speculate that the differences are in fact due to the HA.
4. Subjects will be asked to maintain their normal training regimen throughout the course of the study, however if they decide to stop or change their training regimen the results could be affected.

Significance of the Study

The present study was one of the first studies to examine the use of a HA model to acclimate to hypoxia prior to ascent to altitude. This model could serve as an alternative approach to improve exercise capacity during high altitude exposure.

Previously used methods for inducing acclimatization/acclimation to high altitude are time consuming and require expensive equipment that may not be available. Thus, this HA prior to ascent to altitude could potentially benefit athletes preparing for competition or military personnel preparing to deploy to high altitude terrain.

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CHAPTER 2

This chapter presents a review article, entitled "Does Heat Acclimation Improve Exercise Capacity at Altitude? A Cross-Tolerance Model" which has been accepted for publication by the *Intertnational Journal of Sports Medicine*. It is authored by Ailish White, Roy Salgado, Suzanne Schneider, Jack Loeppky, Todd Astorino, and Christine Mermier. The manuscript follows the formatting guidelines of the journal. Figures and references are provided at the end of manuscript.

Does Heat Acclimation Improve Exercise Capacity at Altitude? A Cross-Tolerance Model

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Abstract

New approaches to induce altitude acclimation in a relatively short timeframe are needed, as it is not practical for many soldiers and athletes to gain access to specialized training facilities. Acclimation to one environmental stressor could enhance adaptation to various other stressors in animals and humans, this phenomenon has been described as cross-tolerance and involves the activation of common protective pathways. The purpose of this review is to discuss possible mechanisms involved in the cross-tolerance between heat and hypoxia. Future data could potentially support the use of a cross-tolerance model as a means for military personnel to prepare for deployment to high altitude terrain, as well as for athletes competing at high altitude.

Introduction

It is common for athletes and military personnel to be exposed to extreme environments including high altitude (1500-3500 meters). However, many of these individuals are not given the time or means to adapt to hypoxic environments prior to ascending to altitude. Prior high altitude acclimatization/acclimation has been shown to be beneficial for athletes and military personnel [5,20,66], but this is not always possible. Thus, alternative training and/or acclimation methods have been examined as a means to maintain or improve performance when ascending to high altitude environments. Some methods previously used to induce altitude acclimatization/acclimation include: "live high: train low" [43], altitude tents, and intermittent altitude exposure (IAE) [5]. However, these strategies can be time consuming and require expensive equipment that may not be available.

It has been suggested that acclimation to one environmental stressor could enhance adaptation to various other stressors in animals [19,35]. This phenomenon has been described as cross-tolerance and involves the activation of common protective pathways [42]. However, cross-tolerance between different environments may not always be beneficial, resulting in either a positive or negative cross-tolerance. In a study examining the cross-tolerance between cold and hypoxia exposure in rats, animals were chronically exposed to either a cold (5°C) or a warm environment (25°C) with daily exposure to hypoxia. Rats exposed to the cold environment had less tolerance to acute hypoxia than those that were exposed to the warmer environment, indicating a negative cross-tolerance between cold acclimation and acute exposure to hypoxia [19]. Others have examined cross-tolerance between repeated cold-water immersions and exposure to

hypoxia in humans [47]. Data suggest that prior cold-water immersion (12°C) reduced sympathetic and cardiorespiratory responses while cycling during acute hypoxia exposure (F_{IO2} = 0.12). These findings further support previous results presented by LeBlanc [41] from an animal model examining cross-tolerance between cold and hypoxia. The authors exposed rats to continuous moderate cold (6°C) versus intermittent severe cold (-20°C) followed by exposure to 9,144 m. Results demonstrated that intermittent severe cold exposure significantly protected rats when taken to 9,144 m compared to continuous moderate cold exposure.

Heat exposure also has been suggested to have positive effects during exposure to a low oxygen environment. Hiestand et al. [33] heat acclimated (HA) sedentary mice to 36-37°C for either 10 or 14 days, and those that were HA survived longer before drowning (anoxia) in water. These results indicated the presence of a positive cross-tolerance between HA and acute exposure to anoxia in mice due to possible circulatory and metabolic adaptations. These may include a redistribution of blood to vital organs and/or tissues, which could decrease oxygen use during drowning. Oxygen requirements following HA are reduced due to decreases in heat production, thus survival time in mice before anoxic death was prolonged. Researchers have examined the development of cross-tolerance between HA and a low oxygen environment by reducing blood flow to the heart [35,42]. Data showed that rats exposed to prolonged HA (30 d) displayed enhanced cardiac work efficiency and performance versus control, and also were provided greater cardioprotection during ischemia and reperfusion. This could be explained by greater ATP preservation following HA, which may be caused by the transition from fast to slow myosin isoforms [38] and delayed acidosis within the

myocardium [35,42]. Thus, HA may improve the protective response to a low oxygen environment [35].

If similar effects are fully supported in humans, this approach could lead to the development of protocols to help individuals prepare for exercise or work during high altitude exposure, and perhaps increase their ability to maintain or improve exercise performance under hypoxic conditions. Thus, the purpose of the following review is to discuss possible mechanisms involved in the molecular and whole body systemic pathways of cross-tolerance between heat and hypoxia. When applied to working or exercising humans, these findings could potentially support the use of a HA model as a means for military personnel to prepare for deployment to high altitude terrain overseas, as well as for athletes competing at higher altitudes. However, due to the limited data available in humans, it is unknown whether cross-tolerance between heat and hypoxia will lead to a positive or negative cross-tolerance.

High Altitude Exposure

Submaximal and maximal exercise performance in humans is impaired during acute exposure to high (2300 m) and extreme (8848 m) altitudes [9,20,49,83]. Reductions in performance have also been observed as low as 580 m in trained men and women [24]. During ascent to altitude, there is a reduction in arterial oxygen pressure, oxygen saturation of hemoglobin (SaO_2), and arterial oxygen content, which leads to reductions in submaximal and maximal exercise performance [20,65] and in some cases, the development of acute mountain sickness [22,61]. Maximal aerobic capacity (VO_{2max}) has been shown to be reduced by 15% at an altitude of 3000 m [20] and 28% at 4000 m [76], which was attributed to a 26% reduction in the arterial oxygen content. Therefore, a

reduction in VO_{2max} results from reduced oxygen availability to the working muscles. In another study, a similar absolute VO_2 (1.5 L/min) was observed at altitude (4300 m) and at sea level during cycling at a fixed power output (100 W). Since VO_{2max} is reduced by ~25% at 4300 m, subjects were exercising at a significantly greater relative exercise intensity (~65% VO_{2max}) compared to sea level (50% VO_{2max}) [51,84]. Maher et al. [48] examined the changes in exercise capacity in well-trained men during prolonged submaximal cycling (≥ 1 hr) at 75% VO_{2max} during acute altitude exposure. The authors concluded that endurance time was not different during acute exposure to high altitude compared to sea level, however, in order for subjects to exercise at the same relative workload used at sea level it was necessary to reduce the absolute workload during high altitude exposure (from 1,063 to 794 kg-m $\cdot$ min^{-1}). Due to the physiological stressors placed on the body during altitude exposure (see Figure 1) [29], researchers have focused on the benefits of high altitude acclimation and/or acclimatization which induces a number of physiological and hematological adaptations that could potentially increase exercise performance [12]. These adaptations include increases in intracellular oxidative enzymes [78], myoglobin concentration [78], and polycythemia [31].

Traditional altitude acclimation and/or acclimatization protocols are very time-consuming, taking approximately 3-4 wks [46]. The first few hours and/or days of hypoxic exposure result in an increase in hematocrit due to plasma volume contraction [67]. In addition, within 2 hrs of hypoxic exposure (3000 m and 4000 m) there is an increase in plasma erythropoietin (EPO) levels [15], which peak after 3-4 days and subsequently stabilize slightly above sea level values [8,62]. The time course of red cell volume expansion depends on the degree of hypoxia; however, its exact time course still

remains unclear [60]. A recent meta-analysis by Rasmussen et al. [60] concluded that red blood cell expansion is slower than initially reported. In order to exert a significant red blood cell expansion, hypoxic exposure must be at an altitude greater than 4000 m for more than 2 wks, however interindividual variability exists across studies. Ge et al. [23] reported varied individual response alterations (-41% - 400%) in plasma EPO following 24 hrs at 2800 m.

Intermittent Altitude Exposure to Maintain Performance at Altitude

The primary goal of simulated altitude training is to improve athletic and occupational performance. Recently, IAE has been used as an alternative to chronic altitude acclimatization as a possible means to improve exercise performance at altitude [5,6,11]. Following 17 days of intermittent hypobaric hypoxia in combination with low intensity exercise, healthy mountaineers were able to properly acclimate and improve aerobic performance[11]. Three weeks of IAE at 4300 m in combination with a rest-work training protocol improved cycling time-trial performance at altitude by 21% with a 10% increase in resting SaO_2 [5]. Beidleman et al. [6] reported that seven days of IAE was sufficient to improve time-trial cycling performance at altitude by 16% versus baseline. The improvement in time-trial performance was likely due to several physiological adaptations that occurred during the constant-work rate exercise trials, including reductions in heart rate (9-20 b/min), VO_2 (1.5-1.6 L/min), and RPE, and a higher SaO_2 (3-4%) compared to baseline at altitude, suggesting that the subjects worked at a lower percentage of VO_{2max} at 4300 m. Overall, IAE elicits physiological adaptations that are consistent with those seen following chronic altitude acclimatization [26] and is effective to improve time-trial performance at altitude [6]. New approaches to rapidly induce

acclimation to high altitude in a relatively short timeframe are still needed, as it is not practical for many soldiers and athletes to access facilities providing IAE. There is a need to develop other strategies that may preserve or improve performance during acute exposure to altitude, and are practical to employ in situations that require short notifications or rapid deployment for athletes and military personnel.

Hypoxic Inducible Factor-1 during Heat Acclimation and its Role in Altitude Acclimation

During acute exposure to a hypoxic environment the body responds by increasing cardiac output and ventilation to attempt to restore oxygen delivery to the body [52,54]. A similar oxygen homeostatic response is also observed at the cellular level accomplished in part through the control of hypoxia inducible factor-1 (HIF-1), which is ubiquitously expressed in all cells and has been described as the master regulator of oxygen homeostasis [71]. HIF-1 transcriptional activity has been associated with the expression of over 70 genes, some of which aid in the adaptive response to hypoxia including enhanced oxygen delivery and/or metabolic adaptations to hypoxia [17,71]. Exposure to a hypoxic environment leads to adaptive responses at the systemic, local tissue, and intracellular level through erythropoiesis, angiogenesis (via vascular endothelial growth factor-VEGF), and increases in glucose transporters and glycolytic enzymes to produce adequate energy in the absence of diminished oxidative phosphorylation [17,70,71].

Recently, researchers have examined the efficacy of cross-tolerance between various environmental stressors, further proposing possible metabolic and molecular mechanisms that could potentially link HA with hypoxia [35,36]. Data from animal

models show that the development of cross-tolerance using HA improved mechanical and metabolic performance of the heart, as well as reduced injury after the heart was subjected to an ischemic insult [42,44]. It has been hypothesized that HIF-1 is associated with HA metabolic responses even in the presence of adequate oxygen [50]. In rats, it has been shown that HIF-1α levels are increased after 30 days of HA and rapidly upregulated during an acute heat shock. In addition, HA upregulates erythropoietin mRNA in the kidneys, erythropoietin receptors in the heart, and VEGF mRNA levels [50]. HIF-1 may be activated via oxygen-independent pathways that aid in improved oxygen delivery after a heat shock exposure, and this response is of a greater magnitude in the HA phenotype [50]. Ultimately, the HIF-1 pathway may contribute to the cross-tolerance between heat and hypoxia and it is possible that this pathway is an underlying element of the HA process [50].

We speculate that adaptation to heat could potentially induce protective responses or have an added benefit during exposure to a hypoxic environment by improving oxygen supply to the body and/or working muscles through upregulation of the HIF-1 pathway. The strategy of using HA to better adapt to hypoxic environments could possibly be applied to maintain or improve the performance for those who travel to high altitudes to work or compete.

Heat Acclimation

Heat acclimation has been shown to induce numerous physiological adaptations that improve aerobic performance including reduced oxygen uptake and blood lactate at a given power output [69,85], muscle glycogen sparing [16,85], plasma volume (PV)

expansion [3,73], improved myocardial efficiency [37], and increased ventricular compliance [39]. Complete HA while engaging in exercise occurs after ~7-10 days of exposure; however ~75% of the physiological adaptations are seen within 4-6 days [57]. HA decay has been under much debate, however it is generally thought that adaptations gained from HA are well maintained for approximately one month in competitive athletes [57].

Lorenzo et al. [45] were the first to demonstrate the impact of 10 days of HA on aerobic performance during a time-trial in a cool (13°C) and warm (38°C) environment. They concluded that 10 days of HA provided substantial ergogenic benefits during exercise in both hot and cool environments. These improvements could be due to an increase in VO_{2max}, which may result from PV expansion, improved myocardial efficiency and increases in ventricular compliance. Heled et al. [32] examined the effect of 12 days of HA on the onset of blood lactate accumulation (OBLA) during an acute exposure to moderate hypoxia (2400 m). They concluded that OBLA following HA was delayed during graded exercise testing in both the normoxic and hypoxic conditions; however, no differences ($p > 0.05$) between OBLA during the two conditions were observed. Following HA maximal aerobic capacity did not change, suggesting that exposure to heat and not a training effect (no change in VO_{2max}) led to increased physiological efficiency during acute altitude exposure. Heled et al. [32] proposed two mechanisms explaining how HA may promote adaptation that could benefit an individual during altitude exposure: 1) HA can reduce metabolic rate during exercise [69], which could contribute to greater efficiency when exposed to high altitude and, or 2) HA increases the expression of HIF-1 [50], which, in its active form, contributes to the

adaptive response during hypoxic exposure by increasing oxygen delivery to the tissues [71]. Thus, there is potential for HA to be used as a strategy prior to ascent to high altitude in order to reduce physiological demands placed on the body when exposed to a hypoxic environment. However, it is unknown whether all individuals will benefit from HA prior to high altitude exposure due to individual variability during heat exposure.

Plasma Volume Loss during High Altitude Exposure

Heat dissipation during exposure to high altitude has been examined, suggesting that convective heat transfer from the skin to the atmosphere is reduced and that evaporative heat loss is enhanced due to the reduced barometric pressure [21,28]. During exercise at altitude, a larger portion of cardiac output is distributed to the working muscles compared to normoxic conditions [74], thus a greater amount of plasma water may be shifted to the working muscles resulting in reductions in PV [18]. Eight months of exposure to altitudes between 4572-5791 m was associated with a reduction in PV of 11-27% [59]. Takamata et al. [77] exposed subjects to hypoxia equivalent to 3400 m by having subjects breathe 13% O_2 during a graded exercise test. Results revealed a larger decrease in PV at a given oxygen consumption during the hypoxic trial compared to the normoxic trial. Alexander and Grover [1] concluded that 10 days at 3100 m is associated with a reduction in left ventricle filling due to a decrease in venous return both at rest and during exercise, which could be explained by a 20% reduction in PV. More recently, Miyagawa et al. [53] examined whether convective heat loss would be decreased due to a reduction in vasodilation during exercise in a warm environment (29-31°C) at high altitude when compared to sea level. Data suggest that cutaneous vasodilation sensitivity is reduced even in the presence of an increased core temperature. This may be due to the

greater exercise-induced PV loss observed during the hypoxic exposure compared to normoxia, which could potentially decrease venous return to the heart [18,55]. In one study, the greater PV loss observed during hypoxia may be due to increased total vascular conductance, which was ~20% higher compared to normoxia (610 m). The authors suggest that the greater PV loss during hypoxia is due to increased muscle vasodilation resulting from increased accumulation of metabolites, ultimately increasing capillary pressure and causing plasma water to shift into the muscle [75].

Plasma Volume Expansion following Heat Acclimation

Heat acclimation can induce PV expansion, which increases aerobic and thermoregulatory capacities [2,13,56,58,73]. Mechanisms responsible for PV expansion following HA include: 1) increased sodium and water retention, which increases the extracellular fluid and PV, 2) increase in circulating protein that increases oncotic pressure and moves water from the interstitium to plasma; and, 3) enhanced dilation of the veins in response to the heat which decreases post-capillary resistance and increase the net fluid movement into the plasma [4,30,68,72].

Lorenzo et al. [45] concluded that following 10 days of HA, power output increased at lactate threshold by 5% during exercise in cool (13°C, 30% RH) and hot (38°C, 30% RH) conditions, maximal cardiac output increased by 9.1% in the cool condition and by 4.5% in the hot condition, and time-trial performance, measured as total work (in kJ) completed within 1 hr increased by 6% in the cool condition and by 8% in the hot condition compared to baseline. An increase in VO_{2max} was also observed, leading the authors to speculate that the increase could have resulted from the increase in PV

(6.5%) [68]. An increase in PV could elicit improved myocardial efficiency and ventricular compliance [39] resulting in an increase in end-diastolic volume. However, controversy exists as to whether expansion of PV can increase cardiac output and whether it consistently results in an increase in VO_{2max} [45].

Acute Plasma Volume Expansion and Exercise Performance

Hopper et al. [34] demonstrated that pre-exercise intravenous infusion of 403 mL of a 6% dextran solution induced PV expansion and increased stroke volume by 11% in untrained men during submaximal exercise. Coyle et al. [14] examined the effects of PV expansion on VO_{2max} and short-term high intensity running performance in untrained men. A larger expansion in PV (500-700 mL) did not improve ($p > 0.05$) VO_{2max} or performance; however, a smaller PV expansion of (200-300 mL) increased VO_{2max} and time to exhaustion by 3.7% and 6.8%, and increased stroke volume by 15%. The authors concluded that the expansion of PV by only 200-300 mL in untrained men may be optimal compared to larger (500-700 mL) PV expansion for improving short-term high intensity running performance. A larger PV expansion causes a much greater hemodilution (~11%) and results in a decrease in VO_{2max} and performance compared to baseline. Thus, there appears be an optimal range for PV expansion to contribute to increases in stroke volume, cardiac output, and a higher peak left ventricular end-diastolic volume during exercise. Grant et al. [25] found that acute PV expansion (low, 14% and high, 21% PV expansion) during 120 min of moderate exercise in a thermoneutral environment increased cardiac output and stroke volume and decreased heart rate. However, this did not attenuate cardiovascular drift during prolonged exercise. Similar findings were also observed during 90 min of cycling at 64% VO_{2max} following a 15.8%

expansion of PV [64]. Acute PV expansion can lead to significantly reduced hematocrit and hemoglobin concentration causing a reduction in arterial oxygen content. However, this did not negatively influence VO_2 kinetics, VO_{2peak}, or time to exhaustion during cycling. In addition, a 14% increase in PV increased VO_{2peak} by 6 % despite an 8% reduction in hemoglobin concentration [7]. However, others have found no change in maximal cardiac output following PV expansion at sea level [40,80]. In moderately trained men, Watt et al. [81] reported no improvement in time trial performance in the heat despite a 13 % expansion of PV.

Calbet et al. [10] examined the effect of PV expansion on exercise capacity and maximal cardiac output in acclimatized lowlanders after spending 9 weeks at 5260 m. In this study, expansion of PV had no effect on VO_{2max} or maximal cardiac output following acclimatization to high altitude. However, Robach et al. [63] examined 31 days of decompression in a hypobaric chamber at an altitude greater than 4500 m and up to 8848 m during the last two days of ascent. Incremental VO_{2max} tests were performed with and without PV expansion at sea level, 6000 m, and upon return to sea level. The authors concluded that acute expansion of PV during a maximal exercise test improved VO_{2max} by 9% at altitude (6000 m) in acclimated subjects.

Several factors could have accounted for the discrepant results observed across studies described above. Watt et al. [81] observed a loss in PV during the early stages of the 40 min exercise bout; this observation was greater during the PV expansion trial. Any added benefits that could have been observed following expansion of PV may have been lost due to the reduction in PV during the submaximal exercise bout. The authors did not expand on the proposed mechanism that may have caused the loss in PV;

however, the increased fluid shifts (i.e., PV loss) observed may be due to the exercise intensity selected (64% VO_{2peak}). Following euhydration and hypohydration, data from men exercising at 22, 37, and 53% VO_{2max} revealed that regardless of the pre-exercise hydration state, exercise intensity was directly related to the magnitude of PV loss during cycling [86]. Cycling exercise can cause hemoconcentration due to an increase in capillary hydrostatic pressure [30], which results from increases in systolic and mean arterial pressure, which are proportional to exercise intensity [27]. Robach et al. [63] did not measure cardiac output, so the authors were unable to determine the exact mechanism that caused an increase in VO_{2max}. Calbet et al. [10] argue that the study by Robach et al. [63] only induced a small expansion in PV, whose magnitude may be unable to enhance VO_{2max}.

The reduction in PV has deleterious effects on both VO_{2max} and hemodynamics typically observed during acute hypoxia, and could potentially be prevented via expansion of PV by incorporating a HA model prior to altitude exposure. In human and animal models, HA has been shown to expand PV and promote increases in stroke volume, cardiac output, and ultimately oxygen delivery to working muscles, thus minimizing cardiovascular strain during exercise at altitude. However, this may not be beneficial for highly trained individuals or athletes who may already have an increased PV, and further expansion may not aid in the maintenance or improvement of performance at altitude.

Improved Cardiac Performance in Response to Heat Acclimation

During exercise, the cardiovascular system provides blood flow to the working muscles; however, during exercise at higher temperatures blood flow is redirected towards the skin in order to maintain heat balance. Simultaneous blood flow regulation between the working muscles and the skin surface creates a challenge for the cardiovascular system. Thus, temperature regulation during heat exposure is associated with reduced cardiac filling and stroke volume accompanied by increases in heart rate to maintain cardiac output and adequate oxygen delivery to the muscles [82]. However, following HA there is an adaptive cardiovascular response, which results in a decreased heart rate and increased stroke volume, suggesting an increased cardiac function. In humans, 10 days of HA increased maximal cardiac output by 2.2 L/min, which accounted for the observed 5% increase in VO_{2max} during exercise in a cool environment [45]. Authors suggest that the increased cardiac output and VO_{2max} could result from the PV expansion (6.5%) following HA. However, an expansion in PV without an expansion of red cell mass may not always lead to improvement in VO_{2max}.

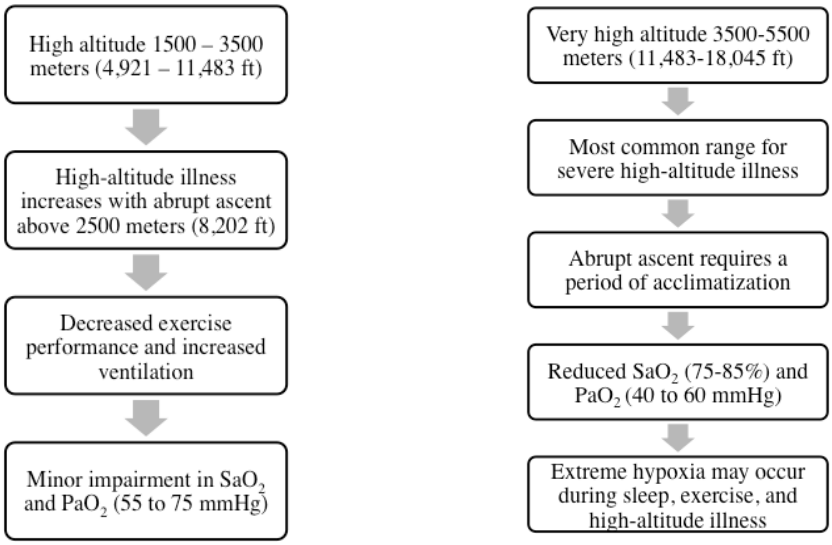
Studies in animal models have demonstrated improved cardiac performance following chronic exposure to heat [37,39,44]. Mechanical differences have been observed between non-acclimated and acclimated hearts by examining the diastolic pressure-volume relationships before and after HA. Results showed that after HA, the heart's diastolic pressure-volume curve is shifted to the right, which allows the ventricle to have a larger filling volume without further increases in filling pressure [35]. Improved cardiac function in a heat-acclimated rat heart is due to increased compliance, contractility, and reduced stiffness of the heart [39]. Horowitz et al. [38] showed that one to two months of HA caused a transition from fast myosin to slow myosin isoenzyme in

rats, which may be due to volume overload of the cardiac muscle and/or a decreased thyroid activity that occurs during HA. Similarly in rats, Levy et al. [44] demonstrated that HA combined with exercise training improved mechanical responses of the heart and overall cardiac performance compared to HA or exercise training alone. Based on these data, the use of HA before ascending to high altitude could potentially reduce cardiovascular strain and improve the function and mechanical properties of the heart. However, the majority of research to date has been done on animal models, while to our knowledge, only one human study has been conducted examining HA and hypoxia, [32], further investigation is warranted [79].

Conclusions

It is unknown whether prior HA could alter exercise capacity during high altitude exposure in humans. However, experiments using animal models suggest that acclimation to one environmental stressor (i.e. heat acclimation) could enhance adaptation to various other stressors (i.e., hypoxia) without any pre-exposure to that specific stressor. Thus, the novel use of a HA model prior to deployment or competition could serve as a possible alternative for individuals as a means to maintain or improve performance during high altitude exposure. The proposed molecular and systemic mechanisms of this model may include: 1) activation of the HIF-1 pathway in which muscle oxygen delivery is improved, 2) expansion of PV leading to increases in venous return and resultant maintenance of cardiac output during exercise at altitude and, 3) improved cardiac efficiency. However, cross-tolerance from one environment to another could result in either a negative or a positive cross-tolerance. Further research is merited to examine this hypothesis. If the data support this HA model, it could be used to pre-

acclimate before high altitude athletic competitions or when military personnel are preparing to deploy to high altitude terrain.



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CHAPTER 3

RESEARCH MANUSCRIPT

This chapter presents a research manuscript, entitled “The Effect of Ten Days of Heat Acclimation on Exercise Capacity during Acute Altitude Exposure (4350 m)". This manuscript will be submitted to the *International Journal of Sports Medicine*. It is authored by Ailish C. White, Roy M. Salgado, Suzanne Schneider, Len Kravitz, Jack A. Loeppky, Todd A. Astorino, James J. McCormick, Trisha A. VanDusseldorp, and Christine M. Mermier. The manuscript follows the formatting and style guidelines of the journal. References are provided at the end of the chapter.

The Effect of Ten Days of Heat Acclimation on Exercise Capacity during Acute Altitude Exposure (4350 m)

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Abstract

Acclimation to one environmental stressor may enhance adaptations to various other stressors in humans; this phenomenon has been described as cross-tolerance. Approaches to induce altitude acclimation in a relatively short time frame are needed, thus the purpose of our study was to examine the effect of heat acclimation on exercise capacity during acute altitude exposure (4350 m). Eight trained men residing at approximately 1600 m performed tests of maximal aerobic capacity (VO_{2max}) at 1600 m and 4350 m, a 16 km time-trial (TT) (4350 m), and a heat tolerance test (1600 m) before and after a 10 d heat acclimation (HA) protocol (40°C, 20% RH). Blood samples were taken at rest pre- and post-HA to estimate changes in plasma volume with HA. Heat acclimation was achieved, as indicated by a significantly lower post-exercise heart rate ($p < 0.01$) and rectal temperatures ($p = 0.42$) on the last versus the first day of HA. Heat acclimation did not alter plasma volume ($p = 0.61$, 1.8 ± 9.9%) or VO_{2max} at 1600 m (4.02 ± 0.58 L/min vs. 4.03 ± 0.43 L/min, $p = 0.88$) or at 4350 m (3.40 ± 0.33 L/min vs. 3.46 ± 0.38 L/min, $p = 0.48$). Time-trial performance was improved by 27.6 sec after HA ($p = 0.06$), which was revealed to be a 95% worthwhile effect according to magnitude based statistics. Our findings do not support the use of HA to significantly improve VO_{2max} during acute altitude exposure. However the improved TT performance suggests that HA was beneficial. Further research with more subjects needs to be conducted to elucidate the use of HA to enhance adaptation to acute altitude exposure.

Introduction

There is growing interest in the participation in mountain recreation and endurance events at high altitude, as well as an increased number of United States troops being deployed to high altitude terrain. Individuals not properly acclimatized/acclimated to high (2300 m) and extreme (8848 m) altitude experience a reduction in both submaximal and maximal exercise performance [13,21,43,55] due to the decline in arterial oxygen pressure, oxygen saturation of hemoglobin, and arterial oxygen content [21,50]. During acute altitude exposure, a specific workload requires a greater percentage of maximal oxygen uptake (VO_{2max}) compared to sea level [44,57]. Moreover, cycling time-trial (TT) performance is reduced by 40% during acute altitude exposure (4300 m) [8] and by 60-70% during intermittent exposure to 4300 m compared to sea level [7,20]. However, others have shown a smaller decrease in submaximal endurance performance following 2-3 weeks of high altitude exposure [31,42]. In addition, Young et al. [58] reported that 15 d of altitude exposure at 4300 m did not significantly change VO_{2max}, similar to findings from Faulkner et al. [17] showing no change following 6 weeks of training at 2300 m. Maximal aerobic capacity may be unchanged from sea level after acclimation, however most athletes and military personnel will be exercising at submaximal intensities. Nevertheless, many athletes and military personnel ascending to high altitude may not be given ample time or means to properly acclimatize/acclimate to a hypoxic environment. Therefore, alternate training and/or acclimation methods have been examined as a means to improve or maintain exercise performance prior to ascending to high altitude environments. Previously used methods include “live high: train low” [37], altitude tents, and more recently, intermittent altitude exposure [7].

However, these methods can be time consuming and require equipment that is expensive or inaccessible.

It has been suggested that acclimation to one environmental stressor could enhance adaptations to various other stressors [19]. This phenomenon has been described as cross-tolerance and involves the activation of common protective pathways [36]. An early study [25] examined heat acclimated (HA) mice at 36-37°C for either 10 or 14 days. Results demonstrated that the HA mice survived longer before drowning. Cross-tolerance between cold and hypoxic exposure has also been examined by exposing rats to either a cold or hypoxic environment. Results showed that rats chronically exposed to the cold environment had less tolerance to acute hypoxia than those that were exposed to hypoxia alone [19]. Consequently, cross-tolerance from one environment to another may not always be beneficial for animals or humans. More recently, researchers have suggested that cross-tolerance may exist between HA and a low oxygen environment in the heart [27]. Following HA in rats, the hearts diastolic pressure-volume curve is shifted to the right, allowing the ventricle to have a larger filling volume without further increases in filling pressure [27]. Improved cardiac function in HA rat heart is due to increased compliance, contractility, and reduced stiffness of the heart [30]. Therefore, we propose that perhaps HA can provide an improved protective response to a low oxygen environment. In humans, improvements in exercise performance following HA result from several physiological adaptations that include reduced oxygen uptake and blood lactate at a given submaximal power output [51,59], increased VO_{2max} [40,52], muscle glycogen sparing [18,59], plasma volume (PV) expansion [5,53], and improved myocardial efficiency [28]. Lorenzo et al. [40] observed an increase in VO_{2max} (+5%) and

cardiac output (+9.1%) in a cool environment (13ºC), as well as a 4.5% increase in cardiac output in the hot environment (38ºC) following 10 days of HA. The authors speculate that the observed increases are due to the 6.5% increase in PV. However, controversy exists as to whether an expansion of PV will lead to an increase in cardiac output and whether it consistently results in an increase in VO_{2max} [40].

More recently, the effects of combined heat stress and moderate hypoxia on exercise performance have been examined [11,22]. Improved high-intensity running performance was observed following heat and hypoxic exposure in elite football players, however, there was no between-group difference in adaptations in the hypoxic and normoxic groups. Interestingly, in the hypoxic group, there appeared to be better maintenance of running performance 4 weeks post-treatment compared to the normoxic group [11]. Girard et al. [22] concluded that acute heat stress in combination with moderate hypoxia (2500 m) reduces cycling time to exhaustion, however, force output of the plantar flexors, muscle activation, and peak twitch torque were similar across all environmental conditions. To our knowledge, only one study in humans has employed a cross-tolerance model to determine the effect of HA on physiological responses during acute altitude exposure in humans [24]. Heled et al. [24] found that 12 days of HA delayed the onset of blood lactate accumulation in both normoxic and hypoxic exercise, and improved cognitive function and dynamic postural tests during hypoxic exposure.

If prior HA could benefit humans ascending to high altitude, it could lead to the development of protocols to be used as an alternative means to maintain and/or enhance exercise performance at altitude. The purpose of the current study was to determine whether 10 days of HA would expand plasma volume and increase VO_{2max} at altitude in

trained men. It was also hypothesized that because of these adaptations, 10 d of HA would improve TT performance at altitude (4350 m).

Methods

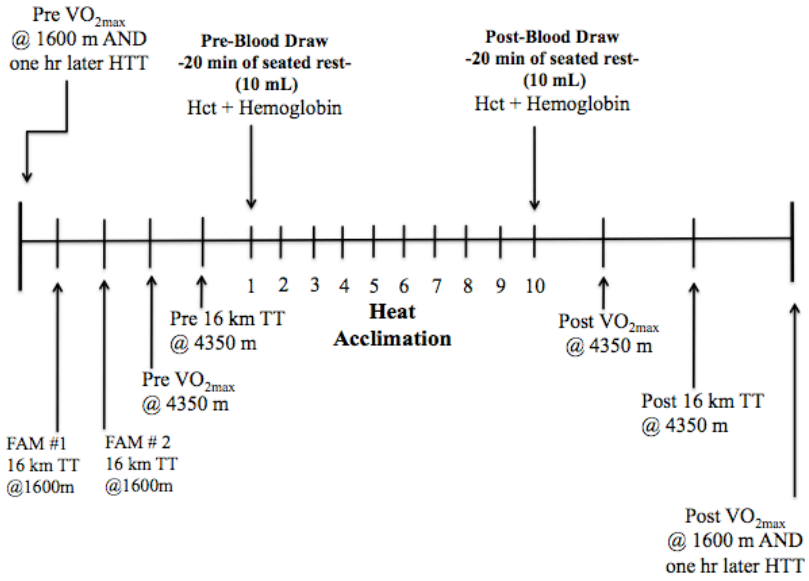
Subjects

Eight trained men were recruited from the University and local communities. The subjects were cyclists and runners, completing 5.9 hrs/wk of moderate and 2.6 hrs/wk of vigorous exercise within the last year. Trained subjects were recruited based on a VO_{2max} of ≥80th percentile for males aged 18-44 yrs [1]. Mean VO_{2max}, age, height, weight, and percent body fat were 53.2 ± 6.7 ± mL/kg/min, 28 ± 5.8 yr, 1.78 ± 0.07 m, 75.2 ± 7.8 kg, and 8.2 ± 3.9 %, respectively. All subjects resided between 1500 and 1600 m for at least the last six months. Subjects were instructed to maintain their normal exercise routines over the course of the study, which was verified with a written physical activity log provided to each subject. All subjects were stratified for cardiovascular risk factors according to the American College of Sports Medicine [1]. Potential volunteers were excluded if they were classified as moderate or high risk. Written informed consent was obtained prior to participating in the study, which was approved by the University's Human Research Review Committee.

Study Design

Subjects reported to the laboratory at the same time of day for each trial and were instructed to refrain from strenuous exercise, caffeine, and alcohol 24 hrs prior to testing. Subjects first completed preliminary testing, which included determination of VO_{2max} and completion of one heat tolerance (HT) test at 1600m, two familiarization 16 km TT at

1600 m and a 16 km TT and a VO_{2max} test at altitude (4350 m). The VO_{2max} test at 1600 m and HT test were completed on the same day and separated by 1 hr, while all other trials were separated by at least 24 hr. Once preliminary testing was complete, subjects reported to the lab to begin a 10 d HA protocol. Following completion of HA, measurements of VO_{2max} and the TT at altitude (4350 m) were repeated and separated by at least 24 hr. The final VO_{2max} and HT test at 1600 m were performed on the same day and separated by 1 hr. Prior to each trial, the subject's nude body weight was recorded and subjects completed a physical activity log. Percent body fat was assessed using a three site (chest, abdomen and thigh) skinfold measurement to estimate percent body fat [33]. Experimental procedures are demonstrated in Figure 1.



Baseline Testing

Measurement of VO_{2max}

Initially, each subject performed a graded exercise protocol in a temperate environment (21°C) to determine VO_{2max}: the first test was performed at 1600 m and the other at 4350 m within a hypobaric chamber. The hypobaric chamber is a custom built chamber, which has an airtight system; it is 6.1 m long and 2.4 m in diameter. A constant flow rate of outside air was used to ventilate the chamber. All testing was performed on an electronically-braked cycle ergometer (Velotron DynaFit Pro, RacerMate, Seattle, WA). The test began at 70 W for one minute, then work rate was increased 35 W every minute until volitional fatigue. Heart rate (HR) was continuously monitored via telemetry (Polar Electro, model FS1, Woodbury, NY), while oxygen saturation (SaO_2) (GO_2 Pulse Oximeter, Philips Respironics, Andover, MA) and rating of perceived exertion (RPE) with a 6-20 scale [9] were measured every minute. The day-to-day error in VO_{2max} determination is approximately 3-4% [4,32]. VO_{2max} was identified as the highest value over 30 s. All gas exchange data were continuously measured using a metabolic cart (ParvoMedics True One 2400, Sandy Utah), which was calibrated pre-exercise according to manufacturer specifications. Per manufacturer's recommendation, the pneumotach flow rate was reconstructed for high altitude trials to account for the reduced air density within the hypobaric chamber.

Heat Tolerance Test

The HT test was performed in a heat chamber at 1600 m and consisted of cycling (Monark Ergomedic, Model 828E, Varberg, Sweden) at a power output equal to ~55% VO_{2max} (158 W) for 45 min at a temperature of 40°C and ~20% relative humidity. Prior to exercise, nude body weight was recorded using an electronic scale (Model 2531, Seca, Danville, VA). Urine samples were collected to determine hydration status via

urine specific gravity (euhydration was classified as ≤ 1.020 g/mL) using a refractometer (REF312ATC, General Tools and Instruments, New York City, NY). If subjects were not euhydrated, they were asked to consume water followed 20 min later by a second assessment of hydration. Once adequate hydration status was determined, subjects were inserted a rectal thermistor (Model 4TH, Telly Thermometer, Yellow Springs, Ohio) ~10 cm past the anal sphincter. Uncovered skin thermistors (YSI 409B, Thermistor Probe, Dayton, Ohio) were placed on the chest, arm, and thigh in order to calculate mean skin temperature (T_{sk}) using an established equation: ($T_{sk} = 0.43\ T_{chest} + 0.25\ T_{arm} + 0.32\ T_{thigh}$) [48]. Rectal temperature (T_{re}) and T_{sk} (Model 44TA, Telly Thermometer, Yellow Springs, Ohio) and HR was recorded every 5 min. Urine output was measured after the trial. Upon completion of the trial, subjects immediately exited the heat chamber and provided a dry nude body weight, which was used to determine whole body sweat rate (WBSR) [12] after correcting for urine loss.

Familiarization Time-trial

Subjects completed a 10 min warm-up at self-selected workload followed by a 16 km self-paced TT (flat course; Velotron RacerMate 3-D Software, Seattle, WA) at 1600 m. This bout was not performed at 4350 m so as not to expose participants to a more severe altitude prior to HA. Subjects were instructed that selecting a higher gear would allow them to attain higher speeds, thus the subjects were allowed to adjust gears manually throughout the entire TT. Water was provided *ad libitum*. Subjects were informed of the distance covered throughout the entire trial and given verbal encouragement; however, subjects were not given any feedback regarding HR, power output, or performance time. Heart rate, SaO_2 and RPE were measured every 1.6 km and

at the end of the TT. Pilot testing revealed a day-to-day error in this measurement of 0.8 % in active individuals, although coefficients of variation as low as 1– 1.5 % have been reported for trained cyclists [3,56]. The second familiarization trial was performed at least 24 hr after the first TT at the same time of day.

Cycling Time-Trial

The TT test was performed in the hypobaric chamber at approximately 454 mmHg (4350 m) [54]. Subjects completed a 10 min warm-up at self-selected workload followed by a 16 km self-paced TT. Subjects were reminded to give an all-out effort and water was provided *ad libitum*. Heart rate, SaO_2 and RPE were measured every 1.6 km and at the end of the TT.

Heat Acclimation

Subjects completed 10 consecutive days of HA at 1600 m in an environmental chamber at a temperature of 40°C and ~ 20% relative humidity. HA was induced using a traditional protocol which consisted of two 50 min bouts of cycling (Monark Ergomedic, Model 828E, Varberg, Sweden) at a power output equal to ~55% of VO_{2max} determined at 1600 m (158 W) [40,46], with 10 min of seated rest between bouts [40,51]. To determine the appropriate exercise intensity during all heat trials, 75 W was subtracted from the workload at ventilatory threshold 1, which was derived from the VO_{2max} test at 1600 m (i.e., corrected workload). The workload was corrected so that subjects exercised at a power output that would elicit a submaximal VO_2 below ventilatory threshold 1 [10] (unpublished data), which has been reported not to elicit a training response [39,40,46,49]. Core temperature was measured using a rectal thermistor integrated to the

Telly Thermometer and T_{re} was recorded every 5 min along with HR and RPE. During the trial, water intake was provided *ad libitum* and the final volume along with urine output were recorded. Nude body weight was recorded before and after each trial to determine WBSR after correcting for urine output and water intake. The HA termination criteria included: 1) completion of the 110 min trial, 2) $T_{re} \geq 40°C$, and 3) subject asked to stop. Exercise time was recorded if subjects were unable to complete the entire 110 min HA trial for any given day.

Prior to exercise on day 1 and day 10 of HA, subjects were seated for 20 min in a cool room [23]. This was followed by a 10 mL venous blood sample drawn from the antecubital vein for the determination of hemoglobin (Hb) and hematocrit (Hct). A small sample of blood was then placed filled into three heparinized capillary tubes and centrifuged (Model C-MH30, Unico, Dayton, NJ) at 12,000 rpm for 5 min in order to determine Hct in triplicate. Blood Hb was measured by Quest Diagnostics (Albuquerque, NM) using established procedures (Beckman Coulter, LH750). The Hct and Hb values were used to calculate plasma volume change using the Dill and Costill equation and corrected for trapped plasma (0.96) [15,16].

Statistical Analysis

Data are reported as mean ± SD and were analyzed using SPSS Version 19.0 (Chicago, IL). Dependent t-tests were used to determine significant differences in end-exercise HR and T_{re} from pre- and post-HA, as well as to determine significant differences between pre- to post-HT test variables, including end-exercise mean T_{sk}, T_{re}, and HR. The change in PV was compared to zero to determine a significant difference

using a dependent t-test. The difference in VO_{2max} and TT performance, as well as end-exercise HR, SaO_2, and RPE in response to HA was compared using a dependent t-test. The level of significance was set at $p \leq 0.05$.

In addition to traditional hypothesis testing, magnitude-based inferences were utilized to examine the "true" effect of this intervention on TT performance [6]. The paired *t*-test *p*-value, mean percent change in performance, degrees of freedom (n – 1), and smallest worthwhile performance benefit (0.3 of the typical within athlete standard deviation; CV for our TT was 1.58 % * 0.3 = ~0.5) were entered into a downloadable spreadsheet (http://www.sportsci.org/resource/stats/xcl.xls) to calculate the ± 95% confidence limits (CLs) and the percent chances that the treatment effect was positive/beneficial (> 75%), negligible/trivial (25-75%), or negative/harmful (< 25 %).

Results

Effect of Heat Acclimation on HR and Temperature

The end-exercise HR and T_{re} decreased from 161 bpm to 140 bpm ($p = 0.01$ and $p = 0.042$) over the 10 d HA protocol, indicating that subjects were HA according to standard criteria (Table 1). Heat acclimation increased PV by 1.85 %, however this was not significantly different from zero ($p = 0.61$) (Table 1). End-exercise mean HR and mean T_{sk} from the HT test were significantly lower ($p < 0.01$ and $p = 0.012$) from pre to post HA, however end mean T_{re} was not significantly different ($p = 0.09$).

Effect of Heat Acclimation on VO_{2max}

Table 2 provides the mean changes in VO_{2max} at 1600 m and 4350 m before and after HA. Data demonstrated no significant differences in VO_{2max} at 1600 m or 4350 m in response to HA (See Table 2). However, VO_{2max} at 4350 m improved by ~2.2% pre to post HA. Individual changes in VO_{2max} at 4350 m are revealed in Figures 3 and 4. Maximal HR, SaO_2, and RPE were not significantly different during the VO_{2max} test at 1600 m and 4350 m before and after HA (Table 2).

Effect of Heat Acclimation on Time-trial performance

There were no significant trends for improved TT performance ($p = 0.06$) and average power output ($p = 0.05$) following HA. Paired *t*-tests comparing changes in TT performance revealed improved performance in response to HA (1749.5 ± 86.3 sec vs. 1721.9 ± 74.7 sec). The mean change in TT performance was -27.6 s and -1.6%, respectively (see Table 3). Individual data (Figure 5) demonstrated greater performance in 7 of 8 subjects in response to HA. Maximal HR, SaO_2, and RPE during the TT were not significantly different before and after HA.

Heat Acclimation		
	Day 1	***Day 10***
Body Weight (kg)	75.2 ± 7.9	75.5 ± 8.4
Final Heart Rate (bpm)	161 ± 17.8	140 ± 15.4*
Final T_{re} (°C)	39.2 ± 0.6	38.7 ± 0.5*
Hemoglobin (g/dl)	15.7 ± 1.6	15.6 ± 0.8
Hematocrit (%)	0.46 ± 0.03	0.45 ± 0.01
Plasma Volume Change (%)	1.8 ± 9.9	

Table 1. Values are shown as means ± SD for 8 subjects on *day 1* and *day 10* of heat acclimation. *$P < 0.05$ for difference between *day 1* to *day 10*.

	1600 m		**4350 m**	
	Pre HA	*Post HA*	*Pre HA*	*Post HA*
VO_{2max} (mL/kg/min)	53.2 ± 6.7	53.7 ± 3.8	45.3 ± 4.1	45.9 ± 3.4
VO_{2max} (L/min)	4.02 ± 0.58	4.03 ± 0.43	3.40 ± 0.33	3.46 ± 0.38
Peak Power (W)	362.4 ± 54.3	374. 3 ± 41.5	321.4 ± 47.8	330.6 ± 44.9
V_{Emax} (BTPS L/min)	156 ± 31.8	169.5 ± 26.7	171.8 ± 37.7	168.2 ± 25.9
HR_{max} (bpm)	173 ± 13.4	178 ± 6.7	169 ± 11.6	167 ± 5.8
SaO_2 (%)	90 ± 2.3	91 ± 4.3	76 ± 3.7	76 ± 3.7
RPE	18 ± 1.7	18 ± 1.3	19 ± 1.1	18 ± 1.1

Table 2. Values are shown as means ± SD for 8 subjects before and after heat acclimation at 1600 m and 4350 m. There was no difference ($p > 0.05$) in the variables displayed in response to heat acclimation.

Comparison	Mean improvement ± SD (s) (95 % CI)	Qualitative outcome (% chances effect is beneficial/trivial/harmful)
Pre-Post TT	27.6 ± 36 (-2.5 to 57.7)	96.5 % beneficial 0.2 % trivial 3.2 % harmful

Table 3. Changes in time-trial performance in response to 10 d of heat acclimation.

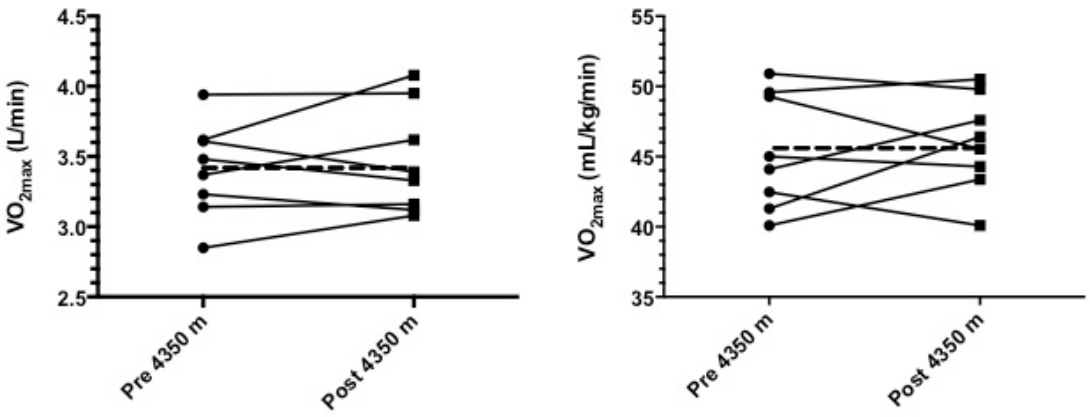


Figure 3. Individual and mean VO_{2max} values (L/min and mL/kg/min) for 8 subjects before and after heat acclimation at 4350 m.

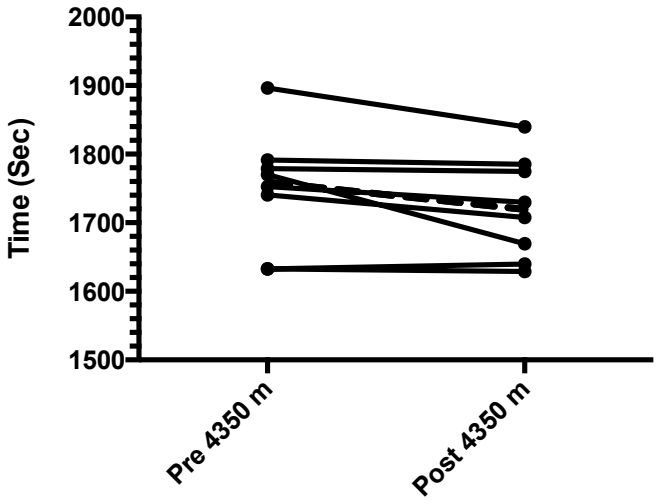


Figure 5. Individual and mean time-trial performance (means ± SD) for 8 subjects before and after heat acclimation at 4350 m.

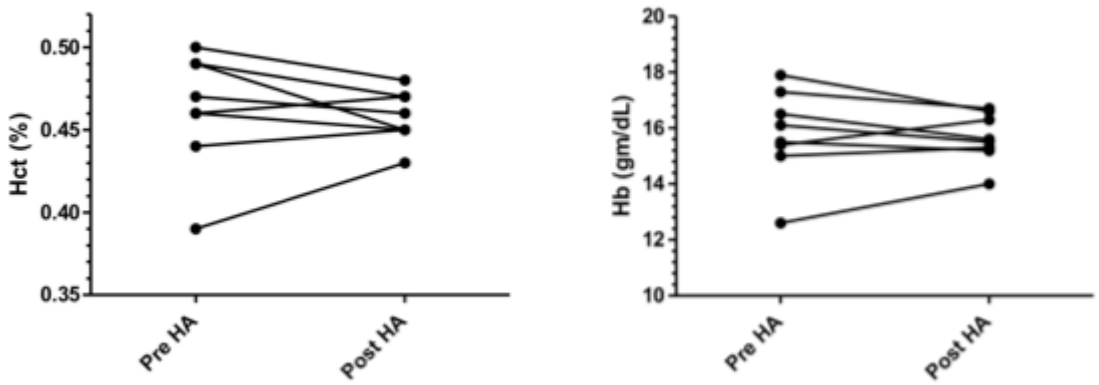


Figure 6. Hematocrit and hemoglobin values shown as means ± SD for 8 subjects before and after heat acclimation.

Discussion

The purpose of the current study was to examine the effects of 10 d of HA on exercise capacity during acute altitude exposure (4350 m) in trained men. Despite the fact that HA reduced HR and T_{re}, no significant difference was observed in VO_{2max} or TT

performance during acute altitude exposure (Table 2 and Figures 3-5) after HA. Yet, the 2.2% improvement in VO_{2max} and 1.6% improvement in TT performance at 4350 m corroborate results from animal and human models showing that exposure to one environmental stressor does not impair performance, and may improve adaptation to another environment [24,25].

Our data do not support previous findings showing beneficial effects of HA on VO_{2max}, however there was a small increase (2.2%) in VO_{2max} at 4350 m. Sawka et al. [52] employed a 9 d HA protocol in trained subjects and reported a 4% increase inVO_{2max} in a moderate environment (21°C), while others have found a similar increase (5%) in a cool environment (13°C) in highly trained cyclists [40]. Sawka et al. [52] concluded that the improvement in VO_{2max} must be due to a "training effect" from the HA protocol. However, their observation is somewhat surprising as the subjects were exercising at ~50% VO_{2max}, which should not elicit a training effect in trained individuals [46]. Lorenzo et al. [40] attributed the 5% increase in VO_{2max} to the observed increase in PV (6.5%) and cardiac output (2.2 L/min). However, in the current study VO_{2max} was not significantly different at 1600 or 4350 m following HA, which is similar to other findings [24,45]. During HA, subjects were exercising below their individual ventilatory thresholds, which should not induce training adaptations [39,49]. It may be that the relatively small observed change in PV (+1.85%) contributed to the lack of improvement in VO_{2max} in response to HA at 1600 m and 4350 m. However, Nielsen et al. [45] found no significant differences in VO_{2max} following HA despite increased PV (+13.1 %) as well as cardiac output and stroke volume. Yet, the authors did not explain the lack of

change in VO_{2max} even in the presence of an increased O_2 delivery and no difference in leg blood flow at 10 min of exercise and at exhaustion.

We proposed that HA would lead to an increase in PV which would increase cardiac output and venous return, ultimately increasing VO_{2max} and TT performance at altitude. Plasma volume loss during altitude exposure (within 5-10 days) reduces stroke volume and cardiac output [2], which can have deleterious effects on hemodynamics, VO_{2max} and performance. This could potentially be prevented via expansion of PV by incorporating a HA model prior to altitude exposure. However, our hypothesis was not fully supported, as VO_{2max} was not significantly different at 4350 m following HA, and we observed a non-significant increase in PV. There is limited research to support these data, as there are few investigations examining effects of HA on VO_{2max} and TT performance during acute altitude exposure (4350 m). However, it is important to note that we did observe a non-significant increase (2.2%) in VO_{2max} at 4350 m, as well as an improvement in TT performance (-27.6 sec) following HA. We speculate that the non-significant improvements in VO_{2max} and TT performance could be due to attenuation in sympathetic nervous activity [26], improved recruitment of slow-twitch muscle fibers [51], and reductions in glycogenolysis following HA [18,59], which would contribute to a reduction in metabolic perturbation. In addition, cellular adaptations may occur in response to heat stress, which may increase mitochondrial biogenesis [38]. In highly trained subjects oxidative capacity is the strongest predictor of performance [34], so it is possible that these peripheral changes occurred in response to HA leading to improved TT performance, although further study is needed to confirm this claim.

To our knowledge, the study of Heled et al. [24] was the first to emply a cross-tolerance model in humans by examining the effect of HA on physiological strain (as measured by OBLA) and cognitive function during acute moderate altitude exposure (2400 m). Results demonstrated that the OBLA was delayed in both a normoxic and hypoxic environment after 12 d of HA, yet there was no change in VO_{2max}, suggesting that exposure to heat and not a training effect delayed the OBLA during acute altitude exposure [24]. However, it is important to note that the altitude stimulus was mild (2400 m) and extremely brief duration (10 min), thus these findings cannot be generalized to real-world settings, as athletes and military personnel sojourn to high altitude for prolonged periods of time.

Heat acclimation was achieved in our subjects, as end-exercise HR and T_{re} were significantly lower from *day 1* to *day 10* (Table 1). We observed a relatively small change in resting PV (+1.85%) following HA, while others have reported, on average, larger increases (5.2-16%) following HA [35,40,41]. Lorenzo et al. [40] attributed the increase in VO_{2max} following HA to the observed increase in PV (6.5%) and cardiac output (2.2 L/min), although this has not exhibited in other studies [40,45]. In one study, Calbet et al. [14] examined the effect of PV expansion on exercise capacity and maximal cardiac output in acclimatized lowlanders after 9 weeks at 5260 m. Their data revealed PV expansion had no effect on VO_{2max} or maximal cardiac output following acclimatization to high altitude. However, Robach et al. [47] examined 31 days of decompression in a hypobaric chamber at an altitude greater than 4500 m and up to 8848 m during the last two days of ascent. Incremental VO_{2max} tests were performed with and without PV expansion at sea level, 6000 m, and upon return to sea level. The authors

concluded that acute expansion of PV during a maximal exercise test improved VO_{2max} by 9 % at altitude (6000 m) in acclimated subjects. The authors were not able to determine the exact mechanism by which PV expansion caused an increase in VO_{2max} at high altitude. However, they hypothesized that in the face of reduced blood volume, an increase in PV could improve venous return and ultimately increase cardiac output and blood flow to the muscles. We speculate that the non-significant change in PV in our subjects could have led to a lack of change in cardiac output and O_2 delivery, thus neither ventricular compliance nor myocardial efficiency would be improved [28,29].

This study has a few important limitations. We did not measure cardiac output, thus we are unable to elucidate why VO_{2max} did not change in response to HA. There is a possibility that implementing familiarization trials at 4350 m instead of at 1600 m, as well as repeating the TT multiple times before and after HA could have given us reduced variability in our TT results. Yet, this would have elicited additional exposure to high altitude, which could have altered resultant adaptations to HA. Our study did not include a control group and we had a relatively small sample size, thus we cannot unequivocally state that a cross-tolerance model between heat and altitude does not exist in humans. Although our subjects were classified as trained, our subjects were not homogeneous. Their varying training status resulted in dissimilar Hct values prior to HA, and the more highly trained subjects tended to have higher Hct values compared to those who were less fit. Perhaps future research should be conducted in subjects with similar training status. Our subjects had been residing at a mild altitude (1600 m) for at least 6 months; therefore we do not know how sea level residents would respond to a similar cross-tolerance model.

In conclusion, previous research shows that HA at sea level can expand PV and increase stroke volume, cardiac output, and ultimately oxygen delivery to working muscles [40,45], leading to enhanced VO_{2max} and/or TT performance during exercise at altitude. Although we did not find significant differences in VO_{2max} at altitude following HA, we did observe an improvement in TT performance, thus HA does not have a detrimental effect on VO_{2max} and TT performance at 4350 m. Additional research is merited to further our findings as a cross-tolerance model has great applications for potential maintenance or improvement of performance at altitude, as well as practical importance for individuals being transported from a hot environment to high altitude.

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CHAPTER 4

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

The review manuscript entitled "Does Heat Acclimation Improve Exercise Capacity at Altitude? A Cross-Tolerance Model" was published in the *International Journal of Sports Medicine,* in January 2014 and adds new insight into the potential use of a cross-tolerance model between heat and altitude. To my knowledge this was the first review manuscript published that examined how heat acclimation (HA) could improve or maintain performance during high altitude exposure in humans. The proposed molecular and systemic mechanisms of this model include: 1) activation of the hypoxic inducible factor-1 pathway in which muscle oxygen delivery is improved, 2) expansion of plasma volume (PV) leading to increases in venous return and resultant maintenance of cardiac output during exercise at altitude and, 3) improved cardiac efficiency. However, it has been shown that cross-tolerance from one environment to another could result in either a negative or a positive cross-tolerance.

The research manuscript entitled "The Effect of Ten Days of Heat Acclimation on Exercise Capacity during Acute Altitude Exposure (4350 m)" provides evidence that prior HA did not significantly alter VO_{2max} at altitude; however, I did observe an improvement in TT performance (1.6%). Thus HA does not have a detrimental effect on VO_{2max} or TT performance at 4350 m. Only one other human study has directly examined the effects of prior HA on physiological variables during acute altitude exposure, therefore, my study provided additional insight on the effects of cross-tolerance in humans.

Conclusions

Previous research has shown that HA can expand PV and increase in stroke volume, cardiac output, and ultimately oxygen delivery to working muscles, thus minimizing cardiovascular strain during exercise. The 2.2% improvement in VO_{2max} and 1.6% improvement in TT performance demonstrated in our study at 4350 m corroborate results from animal and human models showing that exposure to one environmental stressor may improve adaptation to another environment. More research needs to be done to support or refute these findings, as a cross-tolerance model has great implications and use for potential maintenance or improvement of performance at altitude, as well as practical importance for individuals being transported from a hot environment to high altitude.

Recommendations

Very little is known about the cross-tolerance between heat and altitude, thus it is recommended that future studies continue to examine its application and efficacy, in human models. Researchers should (1) examine how cross-tolerance between heat and altitude affects various outcomes in individuals residing at sea-level compared to living at a mild altitude, (2) examine how a cross-tolerance model would affect performance in individuals exposed to a moderate altitude (1600 m-3000 m), (3) identify hemodynamic responses to a cross-tolerance model, (4) use a larger sample size, and (5) compare magnitude of effects in both treatment and control groups in order to have a better understanding of how HA affects exercise performance at altitude.

APPENDICES

A. Combination Informed Consent/HIPPA

B. Flyer

C. Health History Questionnaire

D. Verification of Subject Compliance to Study Guidelines

E. VO_{2max} Data Sheet

F. Time-trial Data Sheet

G. Heat Tolerance Data Sheet

H. Heat Acclimation Data Sheet

The University of New Mexico Health Sciences Center Consent to Participate in Research

The effect of heat acclimation on exercise capacity during acute altitude exposure (13,451 ft)

Purpose and General Information

You are being asked to participate in a research study that is being done by Dr. Christine Mermier, PhD, who is the Principal Investigator, and her associates. This research is being done to evaluate how heat exposure will affect exercise performance during short-term high altitude exposure. You are being asked to participate because you are a male endurance athlete. Approximately 25 people will take part in this study at the University of New Mexico.

This form will explain the study to you, including the possible risks as well as the possible benefits of participating. This is so you can make an informed choice about whether or not to participate in this study. Please read this Consent Form carefully. Ask the investigators or study staff to explain any words or information that you do not clearly understand.

What will happen if I participate?

The recruitment process will be standardized. However, since UNM students and staff may be interested in the study, we will make sure that the recruitment process will not be coercive if this applies to you. For example, the PI, who is a faculty member, will not be involved with recruitment of students. A private room will be used for all interactions between you and study personnel. If you agree to be in this study, you will be asked to read and sign this Consent Form. After you sign the Consent Form, the following things will happen: You will report to the Exercise Physiology lab and/or the High Altitude Chamber on 23 different occasions. You will not be able to perform any strenuous lower-body exercise or consume alcohol or caffeine 24 hours prior to all visits. You will also be asked not to travel to an altitude greater than that of Albuquerque, NM (5000-6000 ft) or be exposed to a Jacuzzi or sauna during participation in the study.

Day One: 1) You will be asked to read and sign the combined consent/HIPAA form, and fill out the health history questionnaire if you're interested in participating in the study. If you do not have any conditions, including elevated resting blood pressure, which would make it unsafe for you to participate, then you will be invited to continue with study measurements. 2) The researchers will measure your height, weight, resting blood pressure, and percent body fat with skinfold calipers. We will measure skinfolds on your chest, abdomen and thigh.

Trial 1 We will measure your maximal oxygen uptake (VO2max). This can also be thought of as your maximal aerobic capacity or fitness level. You will perform this test at 5,250 ft (Albuquerque's altitude). The purpose of the VO2max test at Albuquerque’s altitude (5,250 ft) is to determine if you fit our criteria for aerobic capacity. If testing shows that you do not fit our criteria, your participation in the study will not continue.

However, you will be given the results of your test. This VO2max test will also be used to determine your workloads for subsequent submaximal exercise tests at this altitude.

To determine your VO2max, you will perform a maximal graded exercise test on a bicycle using a protocol that involves easy cycling (70 Watts) for 2 min, then the workload will get harder (by 35 Watts every minute) until you can no longer maintain a cadence of 60 rpm or it gets too hard for you to continue. During the exercise test, you are required to wear a nose clip and breathe through a mouthpiece hooked up to a hose so that all your expired air can be collected and analyzed continuously using a measurement system. You will also have a heart rate transmitter strap around your chest. This test will last between 8 and 12 minutes. The total time commitment for this first visit will be about one hour.

Trials 2 and 3: You will perform two 10 mile cycling time-trials (TT) at 5,250 ft. We want to determine how quickly you can cycle for 10 miles without resting. These two tests will be separated by at least 24 hrs. You will complete an easy 10 min warm-up (75 Watts) followed by a 10 mile self-paced TT. We will show you how to select a higher gear if you want to attain higher speeds. Heart rate will be continuously monitored, while oxygen saturation (SaO2), how much oxygen is saturated in your blood, and perception of effort (RPE), how hard you feel you are working will be measured every one mile as well as at the end of the TT. You will be informed of the distance covered at the 3 mile mark and every 1⁄2 mile thereafter; however, you will not be given any feedback regarding your heart rate, power output, or performance time. The time this test will take will vary depending on your fitness level and power output. It should take approximately 30-40 minutes. The total time for each of these tests will be one to one and a half hours.

Trial 4 Your maximal oxygen uptake (VO2max) will be measured while you are at high altitude (13,450 ft) in a special chamber. A medical doctor will be present during the maximal exercise tests. The chamber simulates high altitude by changing the air pressure, with lower pressure simulating ascent to higher altitude. The chamber is sealed to maintain pressure, but fresh air is pumped in from the outside. It takes about one minute to "ascend" or "descend" 1000 ft of elevation. To determine your VO2max, you will perform a maximal graded exercise test on a bicycle exactly as you did at Albuquerque's altitude. This includes cycling with the mouthpiece and nose clip until your cadence drops below 60 rpm. The exercise test will take between 8 and 12 minutes to complete. There will be several people on the research team in the chamber with you. You will stay in the high altitude chamber only long enough to ascend to 13,450 ft., complete the test, and descend back to 5,250 ft. The total time for this visit will be 45 minutes to one hour.

Trials 5 and 6: We will determine your submaximal (less than your maximal exercise effort) exercise economy and efficiency, which are common factors that are related to sports performance. You will perform 20 min of submaximal exercise on a bicycle at both 5,250 ft (Albuquerque's altitude) and 13,450 ft. One exercise trial will be performed at 5,250 ft and one exercise trial will be performed at 13,450 ft. These two tests will be separated by at least 24 hrs. You will exercise for 10 min at approximately 50% of your peak power output achieved during the maximal exercise test at the respective altitude. You will be asked to maintain a cadence of 80 rpm. In order to maintain the necessary

cadence, you will be provided visual feedback of the digital tachometer on the bicycle. Five minutes into each 10 min bout, you will be set up to breathe through a mouthpiece and nose clip where your expired air will be collected and analyzed continuously using a measurement system. For your comfort, the mouthpiece will be removed after each data collection time-frame. You can choose to drink water whenever you do not have the mouthpiece in your mouth. The high altitude trial will take longer than the Albuquerque altitude trial as the "ascent" and "descent" of the chamber will take approximately 10 minutes each way. The total time required for each visit will be 45 minutes to one hour.

Trial 7: A heat tolerance test (HTT) will be performed at 5,250 ft to determine how well you will be able to tolerate exercising in a hot room. The HTT will be performed in a heat chamber at 104°F. You will exercise on a bicycle at 50% of your 5,250 ft VO2max (this is considered an easy to moderate exercise intensity) for 45 min. Prior to the HTT and after urinating into a container, you will enter a private room to measure your nude body weight on an electronic scale. Your urine sample will be collected to determine your hydration status. If you are dehydrated, you will be asked to consume 16 ounces (500 mL) of water, followed 30 min later by a second assessment of hydration. You will then be instructed how to self-insert a rectal thermistor ~4 inches (10 cm) past your anal sphincter for measurement of your core body temperature during the trial. Skin thermistors will also be taped on your chest, arm, and thigh to measure skin temperature throughout the HTT. Heart rate (HR) will be assessed continuously during the HTT using a heart rate strap that you wear around your chest. The HTT will be terminated if you: 1) request to stop, 2) are unable to sustain the predetermined exercise workload, or 3) attain a core temperature of greater than or equal to 104°F. This trial will take approximately one to one and half hours.

If your core temperature reaches 104□F or you do not feel well, you will be immediately removed from the heat and you will be asked to lie down with your feet elevated. One of your hands will be placed in a cooler filled with ice water. Towels will be dipped in ice water and applied to your neck, face, arms, and legs. A fan will be directed across your chest and will be run at top speed. Elevating your feet will increase blood return to the central circulation, reducing your heart rate. The combination of cold water and circulating air will rapidly reduce your core temperature, which will also reduce your heart rate. Cold water application in combination with fanning is the gold standard of care for combating heat illness. If your core temperature does not start to return to normal values or signs and symptoms of heat illness are not alleviated, you will be escorted to the Student Health Center or the UNM Emergency room for further medical treatment. Our doctor will follow-up with you to see how you are doing.

A final urine sample will be measured following completion of the HTT. The same procedures as described above will be followed in order to assure that you're properly hydrated prior to leaving the laboratory. If you are dehydrated, you will be asked to stay in the lab and drink water until you are hydrated.

Trials 8-17: You will be asked to complete 10 consecutive days of heat acclimation (HA) which consists of cycling in a hot room (heat chamber) at a temperature of 104°F. Acclimation to the heat will be induced using a HA protocol, which consists of easy to

moderate cycling at 50% of your 5,250 ft VO2max for two 50 min bouts with 10 min of seated rest between each bout. Your core body temperature will be measured via self-insertion of the rectal thermistor. Your heart rate will be monitored continuously and recorded every five minutes. You will be provided with room temperature water and allowed to drink water freely throughout the trials. We will strongly encourage you to drink water every 10 min during all exercise bouts. If you need to urinate you will do so in a disposable urine container in order for us to measure your urine output. Before and after each HA session, your nude body weight will be measured in a private room and a urine sample will be collected to determine your hydration status. If you're not properly hydrated before and after exercise in the heat you will be asked to consume 500 mL of water followed 30 min later by a second assessment of hydration. Weight, urine output and water consumed will be used to calculate your sweat rate. The HA protocol will be terminated if you: 1) complete the 100 min of cycling, 2) attain a core temperature greater than or equal to 104°F or, 3) request to stop. If you're unable to complete the entire 100 min for any given HA trial, your completed time will be recorded, and you will be asked to continue reporting to the laboratory as scheduled in order to finish the entire 10 days of HA. These HA trials will take approximately two and half hours each.

On day one and day 10 of the HA protocol, two teaspoons (10 mL) of blood (with a total of 4 teaspoons or 20 mL for the entire study) will be drawn from a vein in your arm for determination of hematocrit (packed red blood cells) and hemoglobin (carries oxygen in your blood). This will be done to calculate changes in plasma volume (fluid portion of your blood). All blood draws and blood analysis will be performed in the Exercise Physiology Lab at UNM. All of your de-identified blood samples will be stored in a freezer in a locked room (#B04) within the Exercise Physiology Facility. These samples will only be accessible to the PI and co-investigators. All blood samples will be destroyed after publication of the manuscript(s), no more than two years from completion of data collection. Your hemoglobin and hematocrit values will be given to you if you are interested.

Trials 18-23: Following completion of the heat acclimation protocol, you will complete the following tests separated by at least 24 hrs: cycling time-trial at 13,450 ft, VO2max at 13,450 ft, 20 min submaximal cycling at 50% VO2max at 13,450 ft, 20 min submaximal cycling at 50% VO2max at 5,250 ft, VO2max at 5,250 ft, and a post-heat tolerance test at 5,250 ft. The final heat tolerance test will be performed to verify that you're heat acclimated. All of the tests will follow the same procedures as described above for the pre-HA testing. The time commitment for each of these six trials will be less than one hour, with the exception of the heat tolerance test, which could take up to one and half hours.

Participation in this study will take a total of 48 hours over a period of 3-4 weeks.

What are the possible risks or discomforts of being in this study?

Every effort will be made to protect the information you give us. However, there is a small risk of loss of privacy and/or confidentiality. All exercise sessions will be conducted by exercise physiologists who are trained in recognizing the signs and

symptoms that require termination of exercise. All study personnel are CPR/AED certified, as well as trained in the laboratory's emergency protocols. Risks associated with maximal exercise testing may include the following: brief feelings of nausea, lightheadedness, muscle cramps, or dizziness during or after completion of exercise. According to the American College of Sports Medicine, the risk of a cardiac event in normal healthy individuals during a maximal exercise test is minimal, 0.0006% (6 in 10,000). Because you're an endurance trained athlete you're accustomed to exercising at a high intensity for prolonged periods of time, the risk will be less.

Exercising at a higher altitude and exercising in a hot room may also make you have brief feelings of nausea, lightheadedness, muscle cramps or dizziness. Exercising in a hot room may make you feel tired and overheated, and exercising at high altitude may also cause you to feel fatigued. During all of the high altitude trials we will be monitoring any signs or symptoms of acute mountain sickness using a validated questionnaire. Symptoms of acute mountain sickness include nausea, headache, high altitude pulmonary and cerebral edema. However, that these symptoms do not develop in healthy people until at least 6 hrs after ascent, even during heavy exercise. You will only be at peak altitude for approximately one hour, therefore, we do not foresee the development of acute mountain sickness. A medical doctor will be present during all maximal exercise tests at high altitude.

Drawing blood may cause temporary pain and discomfort from the needle stick, occasional bruising, sweating, feeling faint or lightheaded, and in rare cases, infection. You may feel embarrassed or uncomfortable placing the rectal probe, however the rectal probe does not pose any additional risk to you. This procedure is necessary in order to monitor your core temperature for safety reasons. This measurement allows us to make sure that your temperature is not getting high enough to put you at risk for heat stroke/heat exhaustion. Heat stroke/heat exhaustion is defined as a core temperature of greater than 104□F that can cause disorientation, dizziness, headache, nausea, and vomiting. Heat stroke/heat exhaustion signs and symptoms as described above occur during prolonged exercise in the heat when your body is unable to properly cool itself by sweating. The risk of death and/or organ damage due to heat illness is not well documented. In high school athletes, non-fatal heat illness occurred in 1.6 per 100,000 athletic exposures. While heat stroke/heat exhaustion is rare when body core temperature is kept below 104 degrees F, there is a small possibility of unknown risks when exercising in the heat below this temperature. During the heat tolerance test and heat acclimation trials we will record your core temperature and sensation of heat every five minutes and will continuously monitoring your core temperature and how you feel. In that time, if a core temperature above 104°F is either observed or recorded we will immediately stop the exercise before you have any signs or symptoms of heat stroke/heat exhaustion. Thus, at any point during the heat trials we will terminate exercise if you achieve a core temperature of 104□F or if you are not feeling well. The necessary procedures as described above will be taken to cool and lower your core temperature. These procedures would be done to lower your core temperature and help you feel better.

You also may be uncomfortable having to refrain from having any caffeine or eating any food before each visit. This study requires a lot of your time, and the timing of each test

is important, therefore you made feel inconvenienced by the required schedule. There are risks of stress, emotional distress, inconvenience and possible loss of privacy and confidentiality associated with participating in a research study.

How will my information be kept confidential?

Your name and other identifying information will be maintained in locked files, available only to authorized members of the research team, for the duration of the study. For any information entered into a computer, the only identifier will be a unique study identification (ID) number. Your health questionnaire, informed consent, and HIPAA will be completed in a private room. We will keep a key that links you with your ID number, but that link will be kept in a locked filing cabinet with access only to the study team. The link will be destroyed after we publish the study results. In no instance will your name be used for any published or presented accounts of the results. All tests will be conducted in private areas in the Exercise Physiology lab located in Johnson Center or the High Altitude Chamber located in Carlisle Gym. The research team will not access any outside information, such as your medical records. Only the paperwork for the current study will be used. Any personal identifying information and any record linking that information to study ID numbers will be destroyed when the study is completed. Information resulting from this study will be used for research purposes and may be published; however, you will not be identified by name in any publications. Urine samples will be destroyed immediately after your hydration status is determined. All of your de-identified (subject # only) blood samples will be stored in a freezer in a locked room (Room # B04, Johnson Center) only accessible to the PI and co-investigators. All de-identified samples will be destroyed after publication of the manuscript(s), no more than two years from the completion of data collection.

Information from your participation in this study may be reviewed by federal and state regulatory agencies, and by the UNM Human Research Review Committee (HRRC) which provides regulatory and ethical oversight of human research. There may be times when we are required by law to share your information. However, your name will not be used in any published reports about this study.

What are the benefits to being in this study?

There may or may not be direct benefit to you from being in this study. However, your participation may help find out how individuals respond to exercise at high altitude following heat exposure. Following completion of the study you will be informed of your results from all cycling tests. The results from the maximal exercise tests and time trials may be beneficial for you as you can use the information for determining an optimal exercise intensity and duration of exercise. This information can allow you to train more effectively and to potentially become more successful in cycling competitions. We will inform you about all of your blood test results, both pre- and post-testing. Our physician will talk with you if any of your blood tests are not within the normal range.

What other choices do I have if I don’t participate?

Taking part in this study is voluntary so you can choose not to participate.

What are the costs of taking part in this study?

The primary cost for participating in this study is your time. If you park on or around the University campus you will be responsible for all parking fees.

Will I be paid for taking part in this study?

For your time and inconvenience you will be paid in the following amounts with three VISA gift cards: $20 at the completion of all pre-testing; $50 after completion of the heat acclimation; $80 when all testing is complete for a total of $150. The last day of your participation, you will be given the last ($80) gift card.

What will happen if I am injured or become sick because I took part in this study?

If you are injured or become sick as a result of this study, UNMHSC will provide you with emergency treatment, at your cost.

No commitment is made by the University of New Mexico Health Sciences Center (UNMHSC) to provide free medical care or money for injuries to participants in this study.

In the event that you have an injury or illness that is caused by your participation in this study, reimbursement for all related costs of care will be sought from your insurer, managed care plan, or other benefits program. If you do not have insurance, you may be responsible for these costs. You will also be responsible for any associated co-payments or deductibles required by your insurance.

It is important for you to tell the investigator immediately if you have been injured or become sick because of taking part in this study. If you have any questions about these issues, or believe that you have been treated carelessly in the study, please contact the Human Research Review Committee (HRRC) at the (505) 272-1129 for more information.

How will I know if you learn something new that may change my mind about participating?

You will be informed of any significant new findings that become available during the course of the study, such as changes in the risks or benefits resulting from participating in the research or new alternatives to participation that might change your mind about participating.

Can I stop being in the study once I begin?

Yes. You can withdraw from this study at any time without affecting your education or employment at the University of New Mexico.

The investigators have the right to end your participation in this study if they determine that you no longer qualify to take part, if you do not follow study procedures, or if it is in your best interest or the study's best interest to stop your participation.

HIPAA Authorization for Use and Disclosure of Your Protected Health Information (HIPAA)

As part of this study, we will be collecting health information about you. This information is "protected" because it is identifiable or "linked" to you.

Protected Health Information (PHI)

By signing this Consent Document, you are allowing the investigators and other authorized personnel to use your protected health information for the purposes of this study. This information may include: height, weight, age, percent body fat, blood pressure, your self-reported medical & exercise history, cycling exercise test results, heart rate, oxygen saturation (SaO2), rating of perceived exertion (RPE), volume of oxygen consumption (VO2), skin temperature, core temperature, respiratory exchange ratio (RER), thermal sensation, Lake Louise acute mountain sickness questionnaire, and subject questionnaire form (exercise and diet log). We will also collect your urine to assess hydration and blood for the measurement of hemoglobin and hematocrit.

In addition to researchers and staff at UNMHSC and other groups listed in this form, there is a chance that your health information may be shared (re-disclosed) outside of the research study and no longer be protected by federal privacy laws. Examples of this include disclosures for law enforcement, judicial proceeding, health oversight activities and public health measures.

Right to Withdraw Your Authorization

Your authorization for the use and disclosure of your health information for this study shall not expire unless you cancel this authorization. Your health information will be used as long as it is needed for this study. However, you may withdraw your authorization at any time provided you notify the UNM investigators in writing. To do this, please send letter notifying them of your withdrawal to:

Christine Mermier, PhD MSC 04 2610 1 University of New Mexico Albuquerque New Mexico 87131

Please be aware that the research team will not be required to destroy or retrieve any of your health information that has already been used or shared before your withdrawal is received.

Refusal to Sign

If you choose not to sign this consent form and authorization for the use of your PHI, you will not be allowed to take part in the research study.

What if I have questions or complaints about this study?

If you have any questions, concerns or complaints at any time about the research study, Christine Mermier, PhD, or her associates will be glad to answer them at 505-277-2658 Monday-Friday from 8:00 am to 5:00 pm by phone. If you would like to speak with someone other than the research team, you may call the Human Research Review Committee (HRRC) at (505) 272-1129. The HRRC is a group of people from UNMHSC and the community who provide independent oversight of safety and ethical issues related to research involving human participants.

What are my rights as a research participant?

If you have questions regarding your rights as a research participant, you may call the Human Research Protections Office (HRPO) at (505) 272-1129 or visit the HRPO website at http://hsc.unm.edu/som/research/hrrc/.

Consent and Authorization

You are making a decision whether to participate in this study. Your signature below indicates that you read the information provided (or the information was read to you). By signing this Consent Form, you are not waiving any of your legal rights as a research participant.

I have had an opportunity to ask questions and all questions have been answered to my satisfaction. By signing this Consent Form, I agree to participate in this study and give permission for my health information to be used or disclosed as described in this Consent Form. A copy of this Consent Form will be provided to me.

_____ _____

Name of Adult Participant (print) Signature of Adult Participant

Date

I have explained the research to the participant and answered all of his questions. I believe that he understands the information in this consent form and freely consents to participate.

_____ _____

Name of Research Team Member Signature of Research Team Member

HRPO #: 13-599 Page 9 of 9 Version: 04/03/2014

APPROVED: 04/01/2014 OFFICIAL USE ONLY EXPIRES: 02/03/2015 The University of New Mexico Institutional Review Board (HRRC)

UNM

WELL TRAINED CYCLISTS needed for research:

The effects of 10 day heat acclimation on exercise capacity at altitude

HRPO# 13-599

The purpose of this study is to investigate the effects of 10 days of exercising in the heat on an individual's ability to exercise at altitude. Total time for laboratory visits is about 48 hours over a 5-6 week period. As a participant in this study you will receive results of your ventilatory threshold, maximal oxygen uptake (VO_{2max}), and average power output during a 10 mile cycling time-trial which may be beneficial for exercise training purposes.

Selection criteria include:

- Male well trained cyclists
- 20 to 44 years of age
- free of cardiovascular disease, acute illness, and lower body injury
- no prior heat injury (such as heat stroke and heat exhaustion)
- residing in an elevation of approximately 5,000 ft (Albuquerque)

If you are interested in participating, please contact:

-OR-

Ailish White
Health, Exercise, and Sports Sciences
ailish15@unm.edu
760-212-6486

Roy Salgado
Health, Exercise, and Sports Sciences
demano@unm.edu
707-580-4076

HEALTH HISTORY QUESTIONNAIRE (RESEARCH ONLY 11/22/13)

Subject #_______________________________ Date___/___/___

*Phone #: home*_________________________ *cell*_______________

Date of Birth ___/___/___ Age___ Gender____ Ethnicity_______ Phone (W)__________________

Primary health care provider and health insurance_______________________________________
(Only for information/emergency contact)
Person to contact in case of emergency: Name_______________________________ phone #_____________

♦♦♦

MEDICAL HISTORY

Self-reported: Height______ Weight______
Physical injuries:____

Limitations____

Have you ever had any of the following cardiovascular problems? Please check all that apply.

Heart attack/Myocardial Infarction	____	Heart surgery	____	Valve problems	_____
Chest pain or pressure	____	Swollen ankles	____	Dizziness	_____
Arrhythmias/Palpitations	____	Heart murmur	____	Shortness of breath	_____
Congestive heart failure	____				

Have you ever had any of the following? Please check all that apply.

Hepatitis/HIV	_____	Stroke	_____	Cancer (specify type)	__________
Rheumatic fever	_____	High blood pressure	_____	Thyroid problems	_____
Kidney/liver disease	_____	Obesity	_____	Total cholesterol >200 mg/dl	_____
Diabetes (specify type)	_____	Asthma	_____	HDL cholesterol <35 mg/dl	_____
Emphysema	_____			LDL cholesterol >135 mg/dl	_____
				Trygylcerides>150 mg/dl	_____

Have you ever suffered from heatstroke or heat exhaustion? Y N

If yes, please explain____

Do immediate blood relatives (biological parents & siblings **only**) have any of the conditions listed above? If yes, list the problem, and family member age at diagnosis.

____

Is your mother living? Y N Age at death______ Cause___________________
Is your father living? Y N Age at death______ Cause___________________

Do you currently have any condition not listed that may influence test results? Y N

Details____

____

Indicate level of your overall health. Excellent ____ Good____ Fair ____ Poor____ Are you taking any medications, vitamins or dietary supplements now? Y N

If yes, what are they?____

Do you have allergies to any medications? If yes, what are they? ______________________________

Are you allergic to latex? Y N

Have you been seen by a health care provider in the past year? Y N

If yes, elaborate ____

Have you had a prior maximal graded exercise test? Y N. If yes, when? ____________ What were the results?

____

Have you ever experienced any adverse effects during or after exercise (fainting, vomiting, shock, palpitations, hyperventilation)? Y N If yes, elaborate. ________________________________

____

♦♦

LIFESTYLE FACTORS

Do you now or have you ever used tobacco? Y N If yes: type ______________

How long? ______ Quantity____/day Years since quitting______________

How often do you drink the following?

Caffeinated coffee, tea, or soda _______oz/day Hard liquor _______oz/wk Wine _______oz/week

Beer _______oz/wk

Indicate your current level of emotional stress. High____ Moderate ____ Low____

♦♦

PHYSICAL ACTIVITY/EXERCISE

Physical Activity

Minutes/Day (*Weekdays*) Minutes/Day (*Weekends*)

______/______ average ______/_____ average

Do you train in any activity (eg. jogging, cycling, swimming, weight-lifting)? Y N

How well trained are you? ____

Have your participated in cycling exercise/training for the last year Y N

If yes, briefly describe your training_____________________________________

____

Vigorous Exercise (>30 Minute sessions)

_________Minutes/hours a week

♦♦

Verification of Subject Compliance to Study Guidelines

My medical status has changed recently. yes ____ no ___

If your status has changed, please list information here.

I recently used a hot tub, sauna or hot room in the past 24 hrs. yes ____ no ____

If 'yes,' briefly describe the temperature and duration of exposure.

I recently went to an altitude >Albuquerque (1600 m) in the past 24 hrs. yes ____ no ____

I have completed strenuous *lower-body exercise* in the previous 24-48 hrs. yes ___ no ___

I am currently sick. yes ___ no ___

I have consumed coffee AND/OR alcohol in the previous 24 hrs. yes ___ no ___

I have fasted for at least 12 hrs. yes ___ no ___

Please record here the amount of food and the volume of fluid ingested in the last 24 hrs…

Breakfast:

Lunch:

Dinner:

Snacks:

Please describe your training within the last 24 hrs including intensity/duration/frequency of physical activity. Keep in mind that this must be MAINTENANCE TRAINING.

VO_{2max}

Subject: _____ **Date:** _____

Trial #: _____ **1600 m** **4100 m** **pre** **post**

Age: _____ yr

Height: _____ cm

Pre Weight: _____ kg

Resting BP: _____ mmHg

3 Site SkF:	
Sum:	

Protocol: _____ Peak Power: _____ Watts

Time (min)	Workload (Watts)	HR (bpm)	SaO2 (%)	RPE	Comments
Rest	0				
1	70				
2	105				
3	140				
4	175				
5	210				
6	245				
7	280				
8	315				
9	350				
10	385				
11	420				
12	455				
13	490				
14	525				
R1	50				
R2	50				
R3	50				

Time Trial Study--Performance Trial

1600 m 4100 m Pre Post

Subject No. _____ Date _____

Age _____ yr Height _____ cm Weight _____ kg

TIME TRIAL PERFORMANCE

10 MIN WARMUP @_____ W; HRend = _____ b/min

Distance (mile)	**HR (bpm)**	**SaO_2 (%)**	**RPE**	**Time (min:sec)**
Warm-up at 5 min				
1.0				
2.0				
3.0				
4.0				
5.0				
6.0				
7.0				
8.0				
9.0				
10.0				
Finish				

Heat Tolerance Test

Subject #: _____ **Date:** _____
Trial #: Pre Post

Hydration (pre): _____ g/mL

Nude Weight: _____ kg
Resting BP: _____ mmHg

Hydration (post): _____ g/mL

Workload: _____ Watts

Time (min)	HR (bpm)	Trec (°C)	Tchest (°C)	Tarm (°C)	Tthigh(°C)	RPE	Thermal Sensation
Rest							
5							
10							
15							
20							
25							
30							
35							
40							
45							
Recovery 1							
Recovery 2							
Recovery 3							
Recovery 4							

Post Weight: _____ kg

Comments: _____

Heat Acclimation

Subject #: _____ **Date:** _____
HA Trial #: _____

Hydration: _____ g/mL
Pre Weight: _____ kg Hydration: _____ g/mL (Retest)
Resting BP: _____ mmHg

Hb: 1) 2) 3) g/dL
Workload: _____ Watts Hct: 1) 2) 3) %

Time	HR (bpm)	Trec (°C)	RPE	Thermal Sensation	Room Temp (°C)	Relative Humidity (%)	Comments
Rest							
5							
10							
15							
20							
25							
30							
35							
40							
45							
50							
5 rest							
10 rest							
5							
10							
15							
20							
25							
30							
35							
40							
45							
50							
Recovery 1							
Recovery 2							
Recovery 3							
Recovery 4							